46th ANNUAL

MAIZE GENETICS

CONFERENCE

PROGRAM

and

ABSTRACTS

11-14 MARCH 2004

HOTEL CAMINO REAL
MEXICO CITY, MEXICO

THE CONFERENCE RECEIVED FINANCIAL SUPPORT FROM:

(listed in descending order of contribution)

- **NSF National Science Foundation USA**
- **ICGEP International Center for Genetic Engineering and Biotechnology, Italy**
- **SAGARPA Secretaria de Agricultura, Ganaderia, Desarollo Rural, Pesca y Alimentación, Mexico**
- **IRD Institut de Recherche pour le Développement, France**
- **Pioneer Hi-Bred International, Inc. : A DuPont Co**
- **CIMMYT Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico**
- **CANAMI Cámara Nacional del Maíz Industrializado, Mexico**
- **BASF Plant Science, LLC**
- **Syngenta: Biotech and Seeds Divisions**
- **Monsanto Corporation**
- **Renessen**
- **Limagrain**
- **Affymetrix**
- **Cropdesign**
- **Paragon Growth Services, LLC**
- **Orion Genomics, LLC**
- **Ceres, Inc**

WE THANK THESE CONTRIBUTORS FOR THEIR GENEROSITY

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General Information

Meals and Beverage Breaks:
All meals will be served in the "Salon del Sol" room, except on Thursday night March 11, when dinner will be served in the Camino Real II Room. Coffee, soft drinks and refreshments will be served in front of the Camino Real Rooms I and II during breaks

Talks and Poster room:
All talks will be in Camino Real Room I; Posters will be in Camino Real Room II. Posters may be hung late Thursday March 11, following Session 1, and should be removed before noon on Sunday March 14.

Informal Meeting places:
In formal meetings may be held in Oaxaca I and Oaxaca II rooms, which are located in front of the Camino Real II room. Rooms can be reserved on a first served basis. Additional rooms are available on request.

Steering Committee:
Please share your suggestions, comments and impressions of the meeting with the Steering Committee: Mike Scanlon, Martha James, Co-chairs ; Daniel Grimanelli, Local Organizer ; Jorge Nieto-Sotelo, Jean-Philippe Vielle-Calzada, Local Hosts ; Wes Bruce, Gunter Feix, Monika Frey, Sarah Hake, Jay Hollick, David Jackson, Patrick Schnable, Lynn Senior; Karen Cone, Treasurer; Mary Polacco, Abstract Coordinator. Members of the steering committee responsible for contacting financial contributors include: Wes Bruce, Karen Cone, Daniel Grimanelli, Jorge Nieto-Sotelo, Lynn Senior, Jean-Philippe Vielle-Calzada

Saturday Evening at the National Anthropology Museum:
Buses to the Museo National de Antropologia in Mexico City will leave at 5.15 pm, Saturday March 13, from the Hotel Camino Real. The visit will include a 60 minute conference in the museum auditorium, followed by a visit of the main rooms at the museum. Drinks and finger food will be served in the central patio of the museum from 7:30 pm to 9:00 pm. Buses returning to the Hotel Camino Real will leave at 9.00. **[Note that dinner on Saturday night will be served in the Salon de Sol of the Camino Real from 9:30 pm -11:00 pm.**

Acknowledgements:
Many thanks go to Trent Seigfried and Mary Polacco for considerable efforts in the assembly and printing of this abstracts program. Thanks to the Missouri Maize Group for their help, to Mike McMullen for preparing the poster, and to Carol Hake for designing artwork included on the poster. The meeting registration was outsourced to the MU Conference Center, and professionally handled by Amy Jarvis. Special thanks go to Dan Grimannelli, Jorge Nieto-Sotelo and Jean-Philippe Vielle-Calzada for their extraordinary efforts and attention to details that made this meeting possible.

Next Maize Genetics Meeting:
The 47th Annual Maize Genetics Conference will be held March 10-13, 2005 at the Grand Geneva, Lake Geneva, WI. The local organizer is Marty Sachs, msachs@uiuc.edu.

Thursday, March 11 **5:30 - 7:00 pm DINNER**

7:00 - 7:15	**Announcements**	Chairs	
Session 1	**EVENING SESSION**	**7:15- 9:15 pm**	Chair: Dave Jackson
7:15 - 8:00	**Ed Buckler , USDA-ARS** *Bridging Genomics and Breeding with Maize Diversity*		
8:15 - 9:00	**Patricia León, Universidad Autónoma de Mexico**, *Glucose Regulation in Plants: A Dissection of a Complex Signaling Network*		
9:15	**Informal Poster Viewing (hang Posters late Thursday Night)**		

Friday, March 12

Session 2	**Developmental Genetics**	**8:30-10:10 am**	Chair: Sarah Hake
8:30-8:45	**Thomas Dresselhaus, University of Hamburg** *Peptide-Mediated Signaling from the Egg Apparatus of Maize*		
8:50-9:05	**Andrea Gallavotti, University of California – San Diego** *Barren stalk1 and the Control of Lateral Meristem Initiation in Maize*		
9:10-9:25	**David Jackson, Cold Spring Harbor Laboratory** *Control of Phyllotaxy in Maize by ABPHYL1*		
9:30-9:45	**Michelle Juarez, Cold Spring Harbor Laboratory** *Adaxial/Abaxial Specification of the Maize Leaf*		
9:50-10:05	**Elizabeth Kellogg, University of Missouri – St. Louis** *Evolution of Genes Related to leafy hull sterile1 in the Grasses*		
10:10-10:25	**Michael Muszynski, Pioneer Hi-Bred International** *knotted1 Modulates Different Hormone Pathways in Maize Compared to Dicots*		
10:30-10:50 am -BREAK WITH BEVERAGES			
Session 3	**Biochemical Genetics**	**10:50-12:25pm**	Chair: Monika Frey
10:50-11:05	**Chun-Hsiang Chang, Pioneer Hi-Bred International** *Expression of Feedback Insensitive Corn Aspartate Kinase in Corn Seed Results in an Increase of Threonine*		
11:10- 11:25	**Jorge Nieto-Sotelo, UNAM** *Relevance of the Structure of the Middle Region in the Evolution of HSP100/ClpB Proteins*		
11:30-11:45	**David Stern, Cornell University** *A Nucleus-Encoded Sigma Factor Targeted to Both Mitochondria and Chloroplasts*		
11:50-12:05	**Bao-Cai Tan, University of Florida** *The Dominant White Endosperm Factor White Cap Encodes the ZmCCD1 Carotenoid Dioxygenase in a Large Multiple Copy Gene Array*		
12:10-12:25	**Manli Yang, The University of Toledo** *The lethal leaf-spot1 (lls1) Protein Which Catalyzes Chlorophyll Degradation is Localized to the Inner Chloroplast Membrane*		

12:30-1:30 PM - LUNCH			
1:30-3:30 PM- POSTER SESSION **Contributors will be at EVEN-NUMBERED Posters** 3:00-3:30 pm - BEVERAGES SERVED			
Session 4	**Sequencing the maize gene space-a progress report**	**3:30-5:35pm**	Chair: Mike Scanlon
3:30-3:40	**Gary Davis, National Corn Grower's Association** *A Grower's Perspective on Maize Research*		
3:45-3:57	**Patrick Schnable, Iowa State University** *An Assembly of the Maize Genome*		
4:02-4:14	**Brad Barbazuk, Donald Danforth Plant Science Center** *Consortium for Maize Genomics - An Examination of Maize Gene Coverage Obtained From Shotgun Sequences Derived From Methyl-filtered and High COT Selection Libraries*		
4:19-4:31	**Agnes Chan, The Institute For Genomic Research** *Consortium for Maize Genomics – Assembly and Annotation of the Filtered Maize Genome*		
4:36-4:48	**Joachim Messing, Waksman Institute, Rutgers University** *High Resolution Physical Mapping of the Maize Genome and Sequencing a Part Thereof*		
4:53-5:05	**Jeff Bennetzen, University of Georgia** *Techniques for Finishing and the Assembly of Gene-Enriched Shotgun Sequence Data into a Linked Archipelago of Beautiful Gene Islands, Beaches and All*		
5:10-5:22	**Pablo Rabinowicz, Cold Spring Harbor Laboratory** *Maize Genome Sequencing By Methylation Filtration*		
5:27-5:35	**Maize Genetics Executive Committee** *Wrap Up*		
6:00-7:30 pm - DINNER			
Session 5	**EVENING SESSION**	**7:30- 9:15 pm**	Chair: Dan Grimanelli
7:30-8:15	**Nancy Craig, Johns Hopkins University School of Medicine,** *The Mechanism of hAT Element Transposition*		
8:30-9:15	**Luis Herrera-Estrella, Centro de Investigacion y Estudios Avanzados del IPN** *Phosphorus Stress Responses in Arabidopsis and Maize*		
9:30	**Informal Poster Viewing**		

Saturday, March 13

Session 6	**Cytogenetics and Transposons**	**8:30-10:10 am**	Chair: Pat Schnable
8:30-8:45	**James Birchler, University of Missouri – Columbia** *Somatic Karyotype Analysis in Maize*		
8:50-9:05	**Olivier Hamant, University of California – Berkeley** *Eludicating the Cohesion Protein Network by Analysis of Maize Mutants*		
9:10-9:25	**Jerry Kermicle, University of Wisconsin** *Cross Incompatibility Between Maize and Annual Mexican Teosintes*		
9:30-9:45	**Cagla Altun, Purdue University** *A New Twist on DNA Repair: Characterization of the Maize Mre11 Gene(s)*		
9:50-10:05	**Akemi Ono, Stanford University** *Epigenetic Silencing of MuDR/Mu Transposon*		
10:10-10:40 am -BREAK WITH BEVERAGES			
Session 7	**Quantitative Traits / Epigenetics /Cell Biology**	**10:40-12:20pm**	Chair: Jay Hollick
10:40-10:55	**Mei Guo, Pioneer Hi-Bred International** *Allelic Variation of Gene Expression in Maize Hybrids*		
11:00- 11:15	**Carlos Harjes, Cornell University** *Advanced Backcross Analysis of Maize / Zea diploperennis: Identification and Verification of Novel QTL with Agronomic Importance in Hybrid Maize*		
11:20-11:35	**Chris Della Vedova, University of Missouri – Columbia** *RNA Silencing of an Endogenous Gene in Maize*		
11:40-11:55	**Jose Gutierrez-Marcos, Oxford University** *ZmMEG1-1 is an Endosperm Transfer Cell-Specific Gene with a Maternal Parent-of-Origin Pattern of Expression*		
12:00-12:15	**Montserrat Pages, Consejo Superior de Investigaciones Cientificas** *Protein Kinase CK2 Modulates Developmental Functions of the Abscisic Acid Responsive Protein RAB17 From Maize*		
12:30-1:30 PM – LUNCH			
1:30-3:30 PM- POSTER SESSION **Contributors will be at ODD-NUMBERED Posters** 3:00-3:30 pm - BEVERAGES SERVED			

Session 8	Maize genetic diversity - exploration, maintenance and applications	3:30-5:00 pm	Chair: Martha James
3:35-3:45	**Major Goodman, North Carolina State University** *Variation in Latin American Maize*		
3:50-4:00	**Steven Smith, Pioneer Hi-Bred International** *Maize Genetic Diversity*		
4:05-4:15	**Maud Tenaillon, Station de Genetique Vegetale, Ferme du Moulon** *A Multilocus Investigation of the Domestication Process in Maize*		
4:20-4:30	**Marilyn Warburton, CIMMYT** *Accessing Useful Diversity from the CIMMYT Maize Genetic Resources Collection*		
4:35-4:45	**Denise Costich, Boyce Thompson Institute for Plant Research** *Exploring Maize Genetic Diversity to Understand Light Response Pathways*		
5:15 pm Buses depart for trip to Anthropology Museum			
6:00-9:30 pm -MUSEUM TRIP (beverages and finger food provided) **Bruce Benz, Texas Wesleyan University** *A Story of Maize: Archaeological Evidence from Mexico*			
9:30 - 11:00 PM-DINNER			

Sunday, March 14

Session 9	Bioinformatics and Genomics	9:00-10:40 am	Chair: Lynn Senior
9:00-9:15	**Bi Irie Vroh, Cornell University** *Global Picture of Linkage Disequilibrium Assessed on Maize Unigene Set in Maize Inbred Lines*		
9:20-9:35	**Jean-Philipe Vielle-Calzada, CINVESTAV** *Simultaneous Prediction of microRNAs and Their Target mRNAs Acting By Translational Repression*		
9:40-9:55	**David Skibbe, Iowa State University** *Genome-Wide Examination of Gene Expression in Developing Maize Anthers*		
10:00-10:15	**Nigel Walker, University of Oregon** *Photosynthetic Mutant Library: Functional Genomics of Chloroplast Biogenesis*		
10:20-10:35	**Michele Morgante, Universiti di Udine** *Extensive cis-Acting Regulatory Variation and Expression Overdominance in Maize: A Molecular Basis for Heterosis*		
10:40	**FINAL ANNOUNCEMENTS**		
10:45	**ADJOURN**		

Abstracts – Talks and Poster Presentations

Plenary Talks

T1	*Ed Buckler*	*Bridging Genomics and Breeding with Maize Diversity*
T2	**Patricia León**	*Glucose Regulation in Plants: A Dissection of a Complex Signaling Network*
T3	**Nancy Craig**	*The Mechanism of hAT Element Transposition*
T4	**Luis Herrera-Estrella**	**Phosphorus Stress Responses in Arabidopsis and Maize**

Developmental Genetics Talks

T5	*Thomas Dresselhaus*	*Peptide-Mediated Signaling from the Egg Apparatus of Maize*
T6	**Andrea Gallavotti**	*Barren stalk1 and the Control of Lateral Meristem Initiation in Maize*
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T15	**Manli Yang**	*The lethal leaf-spot1 (lls1) Protein Which Catalyzes Chlorophyll Degradation is Localized to the Inner Chloroplast Membrane*

Genomics Workshop Talks

T16	*Gary Davis*	*A Grower's Perspective on Maize Research*
T17	**Patrick Schnable**	**An Assembly of the Maize Genome**
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QTL, Epigenetic, and Cell Biology Talks

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Genetic Diversity Workshop Talks

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T34	**Steven Smith**	*Maize Genetic Diversity*
T35	**Maud Tenaillon**	*A Multilocus Investigation of the Domestication Process in Maize*
T36	**Marilyn Warburton**	*Accessing Useful Diversity from the CIMMYT Maize Genetic Resources Collection*
T37	**Denise Costich**	*Exploring Maize Genetic Diversity to Understand Light Response Pathways*

Museum Talk

T38	*Bruce Benz*	**A Story of Maize: Archaeological Evidence from Mexico**

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T43	**Michele Morgante**	*Extensive cis-Acting Regulatory Variation and Expression Overdominance in Maize: A Molecular Basis for Heterosis*

Biochemical Genetics Posters

P1	**Cyrus Abdmishani**	*Correlation and Path Analysis of Grain Yield and its Components in Maize*
P2	**Analilia Arroyo**	*Characterization of the Plastidic Isoprenoid MEP Pathway in Maize*
P3	**Pat Bafuma**	*Characterization of the OPT Gene Family in Rice*
P4	**David Bergvinson**	*Molecular Mapping of QTL for Fall Armyworm Resistance and Associated Traits in a Tropical RIL Population (CML67xCML131)*
P5	**Paula Casati**	*How High Altitude Maize Landraces Respond to Ultraviolet Radiation - Investigation of Different Mechanisms Involved in UV-B Acclimation*
P6	**Berenice Cueva-Torres**	*Proteomic Profiles and Nutritional Properties of Maize Landraces of 'El Bajio'*
P7	**Kristyn Dumont**	*Substrate Specificity of the Rice Peptide Transporter OsPTR1*
P8	**Emily Dunn**	*Comparative Study of Lepidopteron Resistance in Maize Lines through Protein Analysis*
P9	**James English**	*Evolution of an Amine Oxidase for Detoxification of Fumonisins by Gene Shuffling*
P10	**George Heine**	*Functional Characterization of Evolutionary Conserved MYB Domain Residues Using P1 as a Model*
P11	**Robert Holmes**	*Characterization of a Maize Inhibitor of Aflatoxin Accumulation*
P12	**David Moody**	*Characterization of an OPT Type Transporter from Zea mays*
P13	**Christina Murillo**	*Gene Duplication in the Carotenoid Biosynthetic Pathway Preceded Evolution of the Grasses (Poaceae): Implications for Pathway Engineering*
P14	**William Rapp**	*Anthranilate Synthase from Agrobacterium tumefaciens Promotes Increases in Free Tryptophan When Expressed in Plant Seeds*

P15	**Quintin Rascon-Cruz**	*Amarantin Accumulation in Transgenic Tropical Maize Germoplasm*
P16	**Carol Rivin**	*Evolution of Novel Gene Function by Divergent Targeting of Duplicated Gene Products*
P17	**Silvio Salvi**	*An Introgression Library of the Maize Early-Flowering Variety Gasp Flint into B73*
P18	**Paul Scott**	*Transgenic Maize Grain Containing Porcine Alpha Lactalbumin Has Elevated Levels of Lysine*
P19	**Moira Sheehan**	*Phenotypic Analyses of Phytochrome B Single and Double Mutants in Maize*
P20	**Masaharu Suzuki**	*Cloning and Characterization of viviparous15: Application of MuTAIL-PCR, Blast Filtering, and In Silico Subtraction to Identify Candidate Genes*
P21	**Chi-Wah Tseung**	*Biochemical and Molecular Characterization of Maize vp13 Mutants*

Bioinformatics Posters

P22	**Juan Burgueno**	*Spatial Analysis of cDNA Microarray Experiments*
P23	**Terry Casstevens**	*GDPC: The Genomic Diversity and Phenotype Connection: Accessing Data Sources via XML Web Services*
P24	**Evelyn Hiatt**	*MaizeGDB Curation and undergraduate training: can they be symbiotic?*
P25	**Carolyn Lawrence**	*PGROP: the Plant Genome Research Outreach Portal*
P26	**Christopher Maher**	*Identifying microRNAs in Plant Genomes*
P27	**Octavio Martinez**	*MAZORKA: A Fully Automatic Bioinformatics Process for Maize ESTs*
P28	**Donald McCarty**	*Informatics filtering and cluster analysis of MuTAIL sequences: tools for in silico detection and confirmation of transposon tagged mutants*
P29	**Donald McCarty**	*Informatics infrastructure for performing field genetics on a genomics scale*
P30	**Trent Seigfried**	*MaizeGDB: Four Usage Cases*
P31	**Wei Zhao**	*An Update on the Comparative Maps of Maize and Rice in Gramene*

Cell Biology Posters

P32	**James Crowley**	*Study of the High Protein Trait of Maize Using the In Vitro Kernel Culture Model System*
P33	**Adela Goday**	*Interaction of the Plant Glycine-Rich RNA Binding Protein MA16 with a Novel Nucleolar DEAD Box RNA Helicase Protein from Zea mays*
P34	**Jose Gutierrez-Marcos**	*Developing Tools for the Study of Cellular Dynamics During Maize Development*
P35	**Antoine Harfouche**	*Jasmonic Acid and Ethylene Modulate the Activation of Insect Defense Signaling Pathways in Maize*

P36	**Niloufer Irani**	*Novel Regulation of Anthocyanin Pigmentation by Light*
P37	**Agredano Lourdes**	*Regulation of the Expression of TOR and S6rp Kinase in Maize (Zea mays L.)*
P38	**Wojciech Majeran**	*Comparative Proteomics of Mesophyll and Bundle Sheath Plastid Differentiation in Maize Leaves*
P39	**Georgina Ponce-Romero**	*Root Cap-Quiescent Center: A Never Ending Dialog*
P40	**Kan Wang**	*Establishment of Robust Maize Transformation Systems for the Public Sector*

Cytogenetics Posters

P41	**Evgueni Ananiev**	*Comparative Cytogenetic Map of Two Maize Inbreds: Mo17 and B73*
P42	**Lorinda Anderson**	*Recombination Rate, EST Distribution and Gene Clustering along the Physical Structure of Maize Chromosomes*
P43	**Matthew Bauer**	*Organization of Endoreduplicated Chromosomes in the Endosperm*
P44	**Daniel Grimanelli**	*Characterization of the elongate1 Mutant in Maize*
P45	**Lisa Harper**	*What is the Role of the Noncrossover Recombination Pathway in Meiosis?*
P46	**Carolyn Lawrence**	*The Behavior of Abnormal Chromosome 10 in the Monosomic Condition*
P47	**Michael Lee**	*Meiotic Recombination and Stress in Maize*
P48	**Juliana Melo**	*Maize Centromeres: Organization and Functional Adaptation in the Genetic Background of Oat*
P49	**Wojtek Pawlowski**	*Initiation of Meiosis in Maize by ameiotic1*
P50	**Stephen Stack**	*Integrating Genetic Linkage Maps with Pachytene Chromosome Structure in Maize*
P51	**Juan Vega**	*Localization of Large DNA Fragments Transferred into Maize Chromosomes by Agrobacterium Infection*
P52	**Weichang Yu**	*Chromosomal Localization of Transgenes in Maize by Fluorescence In Situ Hybridization*

Developmental Genetics Posters

P53	**Ivan Acosta**	*Dissecting the Mechanisms of Sex Determination in Maize*
P54	**Gerardo Acosta-Garcia**	*Xochiquetzal (XOC), an Arabinogalactan Protein Essential for Female Gametogenesis in Arabidopsis thaliana*
P55	**Kirstin Arthur**	*Characterization of Maize rop2 Mutant Pollen Suggests Multiple Roles for the ROP2 GTPase in Pollen Tube Development*

P56	**Linnea Bartling**	*Mapping of the Allele pt*-McClintock at a Distinct Locus From Pt1*
P57	**Philip Becraft**	*Analysis of Mu-Tagged Empty Pericarp Mutants from the UniformMu Maize Population*
P58	**Wes Bruce**	*Maize CLAVATA3-functional Ortholog*
P59	**Hector Candela-Anton**	*Genetic and Molecular Analysis of the Wavy Auricle in Blade (wab1) and Milkweed Pod (mwp) Mutants of Maize*
P60	**Heather Cartwright**	*Pangloss Genes are Required for the Asymmetric Divisions of Subsidiary Mother Cells in Maize Stomata*
P61	**Prem Chourey**	*Evidence of Programmed Cell Death and its Possible Role in the Functional Activation of Placento-Chalazal Layer in the Pedicel Tissue of Developing Maize Caryopsis through Maternal-Filial Interaction*
P62	**George Chuck**	*Microarray Analysis of the Branched Silkless Mutant of Maize and the Frizzy Panicle Mutant of Rice*
P63	**Ryan Dierking**	*Identification of Genes Associated with Root Architecture Under Water Stress in Zea mays L.*
P64	**Ana Elena Dorantes-Acosta**	*Molecular and Genetic Analysis of Mutants Causing Male Gametophytic Lethality in Arabidopsis thaliana*
P65	**Andrew Doust**	*Control of Branch Architecture in Foxtail Millet (Setaria italica)*
P66	**Andrea Eveland**	*ABA Sensing Mediates Expression of Vacuolar Invertase during Female Reproductive Development in Maize*
P67	**Diego Fajardo**	*Molecular and Genetic Analysis of rgh Endosperm Mutants*
P68	**Suneng Fu**	*Clonal Mosaic Analysis Revealed Distinct Functions of EMPTY PERICARP2 in Maize Shoot Development*
P69	**Stewart Gillmor**	*Dominant Non-Reduction Mutants of Maize*
P70	**Jose-Luis Godinez-Martinez**	*Differential Expression of the Actin Gene mac1 in the Embryo and Endosperm During Maize Seed Development*
P71	**Jose Gutierrez-Marcos**	*The Globby1-1 (glo1-1) Mutation Affects Cell Proliferation and Differentiation During Early Endosperm Development*
P72	**David Henderson**	*Ragged Seedling2 Leaves Fail to Expand Despite Retention of Adaxial/Abaxial Polarity*
P73	**Wilson Huanca-Mamani**	*INVUNCHE, An ISWI-like Chromatin Remodeling Factor Essential for Megagametogenesis and Early Seed Development in Arabidopsis thaliana*
P74	**Jiabing Ji**	*The Maize Duplicate Gene Narrow Sheath2 Encodes a Conserved Homeobox Gene Function in a Lateral Domain of Shoot Apical Meristems*
P75	**Sharon Kessler**	*Interactions Between XCL1 and KNOX Genes: A Hormonal Connection*

P76	**Katherine Krolikowski**	*Mutations in the MADS Box Genes ZMM8 and ZMM14 Are Associated with an Indeterminate Floral Apex Phenotype*
P77	**China Lunde**	*The Role of the Maize Gene, Thick Tassel Dwarf1, in Inflorescence Architecture*
P78	**Enrico Magnani**	*A Reverse Genetic Approach to Find New Members of the ERF Family of Transcription Factors Involved in Maize Inflorescence Development*
P79	**Mihaela Luiza Marton**	*The Egg Apparatus-Specific Peptide ZMEA1 From Maize is Required to Guide the Pollen Tube Towards the Female Gametophyte*
P80	**Marina Nadal**	*Corn Smut Induced Maize Genes*
P81	**Nasim Sadeghian**	*Cloning Extended auricle1, an Essential Component in Maize Leaf Development*
P82	**Stefanie Sprunck**	*Gene Expression Profiles from Isolated Egg Cells and Pro-Embryos of Wheat*
P83	**Rosalinda Tapia-Lopez**	*Analysis of the Expression Pattern and Regulation and Probably Function of AGL12, a MADS-Box Gene Involved in Development of Arabidopsis thaliana*
P84	**Pilar Tellez**	*Obtainment and Molecular Characterization of Transgenic Cuban Maize Highly Resistant to Spodoptera frugiperda Smith Attack*
P85	**Elene Valdivia**	*Beta-Expansins in Maize Pollen: Role of Zea m 1 in Development and Fertilization*
P86	**Vanessa Vernoud**	*OCL Genes Are Involved in the Determination of Kernel Size in Maize*
P87	**Cunxi Wang**	*Dynamics of Aleurone Cell Formation: The Surface Rule*
P88	**Clinton Whipple**	*Assessing the Functional Redundancy in the Maize C-Class Control of Carpel and Stamen Identity*
P89	**Katrin Woll**	*Isolation of the New Root Mutant rum1 Affected in Lateral and Seminal Root Initiation*
P90	**Michael Zanis**	*Fate and Consequence of the ZAG1/ZMM2 Gene Duplication Across the Grasses*
P91	**Andres Zurita-Silva**	*Genetic Analysis of Root Responses to Phosphate Starvation in Arabidopsis thaliana (L.) Heynh*

Epigenetics Posters

P92	**Karen Cone**	*Chromatin Genes: Discovery, Mutagenesis, and Function*
P93	**Guillermo Corona**	*Role of Chromatin Remodelling Factors During Female Gametogenesis in Arabidopsis thaliana*
P94	**Olga Danilevskaya**	*Imprinting of the Maize Endosperm Specific Gene fie1 Is Mediated by Demethylation of Maternal Complements*
P95	**Stephen Gross**	*Epigenetic Stability at the Maize pl1 Locus*

P96	**Shawn Kaeppler**	*Analysis of Tissue Culture-Induced White Cob Mutants Define Mechanisms of Epigenetic Change Induced by Stress*
P97	**Mary Ann McGill**	*RNAi-Mediated Silencing of Maize Chromatin Genes and Their Effects on Maize Transformation and Genomic Methylation*
P98	**Susan Parkinson**	*Rmr6 Functions in Paramutation and Developmental Epigenetics*
P99	**Michael Robbins**	*Ufo1 Induces Global Gene Up Regulation in Maize Pericarp*
P100	**Rajandeep Sekhon**	*Genetic and Molecular Characterization of Interaction of Different Alleles of p1 with a Dominant Epigenetic Modifier Ufo1*
P101	**Alan Smith**	*CpNpG Methylation Reduction in Plants Homozygous for the Chromomethylase Mutant Allele zmet2:m1 Is Sequence Dependent*
P102	**Nathan Springer**	*The Maize Polycomb Group Gene, Mez1, Shows Imprinted Expression Throughout Endosperm Development*
P103	**Maike Stam**	*Paramutation: Long-Range Epigenetic Interactions in Maize*
P104	**Christopher Topp**	*Centromeric RNAs are a Component of Maize Centromeric Chromatin*
P105	**Virginia Zaunbrecher**	*Allelic Effects of Maize Chromomethylase Mutants on DNA Methylation*

Genomic Structure & Synteny Posters

P106	**Hank Bass**	*Cytogenetic Mapping of Maize with Sorghum BAC FISH Probes*
P107	**John Bowers**	*High-Throughput Anchoring Of Bac-Based Physical Maps Of Maize to Sorghum, Rice And Sugarcane*
P108	**Jennifer Jaqueth**	*High-Resolution Genetic Mapping of Chromosome 1 in Maize after Ten Generations of Recurrent Intermating in the IBM Population*
P109	**Wade Odland**	*Chromosomal Relationships Defined by Repetitive Sequence Profiles*
P110	**Mary Polacco**	*IBM Neighbors -- Mutual Enhancement of Genetic and Physical Maps*
P111	**Erik Vollbrecht**	*Comparative Analysis of Ramosa1 Gene Runction in Maize, Sorghum and Rice*
P112	**Roger Wise**	*Comparative Analysis of a One-Megabase Sequence Spanning the Maize Rf1 Fertility Restorer with the Rice Genome*

Genomics Posters

P113	**Baldomero Alarcon-Zoniga**	*Integrative Genomic Analyisis in Mexican Forage Maize*

P114	**James Allen**	*Dynamic Nature of the Integration of Plastid Sequences into the Mitochondrial Genome*
P115	**Joseph Bedell**	*The Effectiveness of GeneThresher ™ Methylation Filtering Technology in Sorghum and Its Comparison to Maize*
P116	**Carletha Blanding**	*Identification of Early Expressed Genes and Genes Expressed Differently in B73 and Mo17 after UV Radiation*
P117	**Carlos Calderon-Vazquez**	*Construction of Libraries and Analysis of ESTs From a Phosphorus Efficient-Zea mays Line Grown Under Low-Phosphorus Stress*
P118	**Ed Coe**	*Integration of Genetic and Physical Data in 2,585 Contigs*
P119	**Guillermo Corona**	*EST Sequencing Efforts at CINVESTAV-Irapuato*
P120	**Jeremy Edwards**	*Polyphyletic Origins of Cultivated Rice from Pre-Differentiated Ancestors*
P121	**Fulgencio Espejel**	*Host Effects of a Susceptible and a Resistant Maize Line on the Replication and Movement of the Sugarcane Mosaic Virus*
P122	**Christiane Fauron**	*Sequence Comparisons of Six Mitochondrial Genomes From Maize and Teosinte*
P123	**Jack Gardiner**	*Long-Oligonucleotide Arrays in Maize for Comprehensive Analysis of Gene Expression*
P124	**Luca Gianfranceschi**	*Generating a Pollen Functional Map Using Oat-Maize Addition Lines*
P125	**Jose Luis Goicoechea**	*An Integrated Genetic and Physical Map of the Maize Genome*
P126	**Angela Hayano**	*Analysis of Subtractive and Standard cDNA Libraries Produced from mRNA of Drought-Stressed Maize (Zea mays L.) Plants*
P127	**Stephen Howell**	*Gene Expression Patterns during Somatic Embryogenesis in Maize Tissue Culture*
P128	**Christina Ingvardsen**	*Identification of Genes Differentially Expressed in Association with SVMV Resistance in Maize by Combining SSH and Macroarray Techniques*
P129	**Michael Kolomiets**	*Genomic Characterization of the Maize 12-Oxo-Phytodienoic Acid Reductases*
P130	**Susan Latshaw**	*Candidate Gene Selection and Molecular Analysis of 50 smk Mutants*
P131	**Bailin Li**	*An Integrated Physical, Genetic and EST Map of Maize*
P132	**Jose Lopez-Valenzuela**	*Identification of Genes Coordinately Expressed with eEF1A in Maize Endosperm*

P133	**Donald McCarty**	*High-Throughput Insertional Mutagenesis of Genes Controlling Seed Development*
P134	**Valèrie Mèchin**	*A Two Dimensional Proteome Map for Maize Endosperm Development Studies*
P135	**Steve Moose**	*Gene Discovery for Maize Seed Composition and Nitrogen Metabolism Traits using the Illinois Protein Strains*
P136	**Antonio Oliveira**	*Use of an Indica Rice Mutant Collection as a Tool For Root Functional Genomics*
P137	**Enrico Pè**	*Comparison of Transcription Levels in Immature Ears Between Inbred Lines and Corresponding F1 Hybrid By DNA Microarray Technology*
P138	**Bela Peethambaran**	*Comparative Proteomic Analysis of Maize Silks in Aspergillus flavus Resistant and Susceptible Inbreds*
P139	**Ronald Phillips**	*Oat-Maize Addition and Radiation Hybrid Lines: Development and Application*
P140	**Michaela Sauer**	*Transcriptome and Proteome Wide Analysis of Crown Root Initiation in Maize*
P141	**Nathan Springer**	*Optimizing Conditions for SNP Detection Using Oligonucleotide Microarrays*
P142	**Clifford Weil**	*Status of the Maize TILLING Project*
P143	**Katia Wostrikoff**	*Functional Genomics to Understand Chloroplast Gene Regulation*
P144	**Xiaolan Zhang**	*Global Expression Analyses of Genes Involved in Meristem Organization and Leaf Initiation*
P145	**Robert Ziegler**	*The Challenge Program for Unlocking Genetic Diversity in Crops for the Resource-Poor*

Quantitative Traits & Breeding Posters

P146	**Peter Balint-Kurti**	*Towards the Detailed Analysis of QTLs for Southern Leaf Blight and Gray Leaf Spot Resistance*
P147	**Andrew Baumgarten**	*Identification of QTLs Controlling Ustilago maydis Resistance in Two Recombinant Inbred Populations*
P148	**Patrick Brown**	*Population Genetic Analysis of Candidate Genes for Variation in Sorghum Panicle Architecture*
P149	**Dana Bush**	*QTL Analysis of Stomatal Density*
P150	**Letizia Camus-Kulandaivelu**	*A Comprehensive Study of Genetic Structure in a Collection of American and European Maize Inbred Lines and Its Use in Association Genetics*
P151	**Maria de la Luz Gutierrez-Nava**	*Identification of Key Genes for Drought Tolerance in Tropical Maize*
P152	**Hiroyuki Enoki**	*Identification of Quantitative Trait Loci Controlling Early Flowering of a Northern Flint Maize Inbred Line*

P153	**Jenelle Frost**	*The Genetic Basis of Recurrent Selection Gains for Maysin in Maize Silks*
P154	**Silverio Garcia-Lara**	*QTL Analysis for Maize Weevil Resistance in Tropical Maize*
P155	**Michael Gerau**	*Identification of QTL Associated with Root Architecture Under Well-Watered, and Water-Stressed Conditions in Zea mays*
P156	**Philippe Herve**	*How is the TraitMill™ platform delivering valuable target genes for cereal breeding?*
P157	**Fidel Marquez-Sanchez**	*Comparison of "Z" Lines With "PL" Open-Pollinated Inbred Lines of Maize, With Initial Inbreeding of One Half*
P158	**Enrico Pè**	*Identification of QTLs For Heterosis Using Two Pseudo-Backcross Populations in the B73 x H99 Background*
P159	**Froylan Rincon-Sanchez**	*Agronomic Performance of Maize Populations Developed by Different Crop Management and Selection Schemes*
P160	**Valeriy Rotarenco**	*Breeding Effect of Selection at the Level of Haploid Sporophyte in Maize*
P161	**Valeriy Rotarenco**	*Utilization of Maize Haploid Plants in Recurrent Selection Procedure*
P162	**Juan Salerno**	*Identification of Quantitative Trait Loci (QTLs) Conferring Resistance To Mal de Rayo Cuarto (MRC) Virus in Maize*
P163	**Silvio Salvi**	*Root-ABA1, a QTL Affecting Root Angle and Lodging, Leaf ABA Concentration and Other Traits in Maize*
P164	**Ashish Srivastava**	*Heterosis and Combining Ability of CIMMYT and NARS Lines*
P165	**Stephen Szalma**	*Quantitative Trait Locus Mapping of Agronomically Important Traits in Maize with Near Isogenic Lines*
P166	**William Tracy**	*Historical and Biological Bases of the Concept of Heterotic Patterns in 'Corn Belt Dent'*

Transposable Elements Posters

P167	**Rao Abbaraju**	*Diagnosis of Hot Spots for Mu Integration in the Maize Genome: A Progress Report*
P168	**Cesar Alvarez-Mejia**	*Establishing an Ac/Ds-Based Enhancer Detection and Gene Trap System in Maize*
P169	**Ling Bai**	*Manipulation of Lycopene-b-cyclase in Maize*
P170	**Liza Conrad**	*Development of a Two-Component Activator/Dissociation Tagging System in Maize*
P171	**Andres Estrada-Luna**	*Establishing an Activation Tagging System in Maize (Zea mays L.) Through a Modified Suppresor/Mutator Mobile Element*

P172	**Ericka Havecker**	*The Capsid of a Novel Maize Retrotransposon Interacts with Light Chain 8, a Protein with Diverse Roles in Molecular Trafficking*
P173	**Judith Kolkman**	*Regional Activator (Ac) Mutagenesis in Maize*
P174	**Shailesh Lal**	*Discovery of Helitron Type Transposable Elements in Maize*
P175	**Damon Lisch**	*Epigenetic Modification of Mu Activity*
P176	**Damon Lisch**	*Taming the Mutator System*
P177	**Cathy Melamed-Bessudo**	*Retrotransposon Activation in Nascent Polyploids and Silencing in Subsequent Generations*
P178	**Darren Morrow**	*Initial Results from Analysis of the RescueMu Transposon-Tagging Gene Discovery Strategy in Maize and Continuing Research*
P179	**Thomas Peterson**	*Transposon-Induced Deletions: A New Tool for Plant Genomics Research*
P180	**Tony Pryor**	*Activation Tagging for Rust Resistance in Maize and Barley*
P181	**Fabiola Ramirez-Corona**	*Detection of Maize MuDR Transposon of the Mutator Family in the Races Bolita and Zapalote Chico from Oaxaca (Mexico) as a Tool for Gene Flow Monitoring*
P182	**George Rudenko**	*Transposases Controlling Mutator Activity*
P183	**R. Keith Slotkin**	*Post-Transcriptional and Transcriptional Gene Silencing of the Mutator Transposable Element Family by Mu Killer*
P184	**Udo Wienand**	*Development of a Promoter Trapping System in Zea mays L. Using the Transposable Element Mutator and Regulators of the Anthocyanin Biosynthesis*
P185	**Margaret Woodhouse**	*The mop1 (Mediator of Paramutation1) Mutation Exhibits Maternally-Dependent Reactivation of Silenced Mutator Transposons in Maize*

Plenary Sessions

Thursday, March 11 – 7:15 – 8:00 PM

Bridging Genomics and Breeding with Maize Diversity

Edward Buckler
USDA-ARS

T1

Converting the results of plant genome studies into improved breeding strategies remains a major goal of plant genetics. The tremendous natural diversity in maize provides a critical resource towards this achievement, as it can be used for both functional genomics and the basis of effective marker-assisted breeding. In our ongoing effort to characterize molecular and functional diversity in the maize genome, we have developed germplasm and analysis tools to carry out high-resolution association studies in maize. These association approaches have proven effective in identifying potentially useful nucleotide polymorphisms in genes involved in flowering time and kernel quality. However, while association mapping in maize provides high resolution, this approach can result in less statistical power. To exploit both high resolution and statistical power, we will collaborate with several other groups to develop a platform for the dissection of complex traits in maize by utilizing both association and linkage based approaches.

Thursday, March 11 – 8:15 – 9:00 PM

Glucose Regulation in Plants: A Dissection of a Complex Signaling Network

Patricia León; Guevara, Arturo; San Román, Carolina; Arroyo. Analilia; Cordoba, Elizabeth; Dupreé Patricia; and Cortéz, Ma. Elena
Instituto de Biotecnología UNAM

T2

Glucose acts as a signaling molecule that affects cellular processes in diverse organisms including bacteria and humans. Plants have evolved a complex sugar detection system that involves several signaling pathways. Through these signaling pathways, the expression of a vast number of genes involved in diverse processes such as embryogenesis, seedling development, root and leaf differentiation, etc. are regulated. In an effort to identify components of the glucose signaling pathways from plants, we have isolated mutants insensitive to high glucose concentration (*gin*) in *Arabidopsis*. The isolation and characterization of such mutants, has permitted to uncover a complex network that links sugar signaling to the abscisic acid (ABA) phytohormones.

Several of the genes isolated from our screen participate in the biosynthesis or signaling of the ABA. The mutants *gin5* and *gin8* both affect ABA levels. In addition, the characterization of *gin6* and *gin9* mutants, revealed their allelism to the ABA responsive mutants, *abi4* and *abi5*. *ABI4* and *ABI5* encode two transcription factors of the *APETALA 2* (AP2) and the basic leucine zipper (bZIP) domain families. We have confirmed that the expression of *ABI4* and *ABI5* genes are modulated by multiple signals, including glucose and osmotic. The specificity of the glucose regulation over these genes has been demonstrated by kinetic studies and by the use of sugar analogs, which acts as a sugar signal but not as an osmotic stimulus. More recently it was found that the *cis*-acting regions involved in glucose and osmotic regulation of the *ABI4* are located in a different part of the gene. Also it has been found that ABI4 acts a positive enhancer of its own transcription, but the induction by glucose is still observed in its absence. A similar positive feedback regulation by glucose was also found with several of the ABA biosynthetic genes. Thus, glucose has the capacity to modulate the expression of the genes that participate in its

own signaling. Transcription factors such as ABI4 and ABI5 that are regulated by a variety of signals, might contribute to the ability of plants to respond in a flexible and integral way to continuous changes in the environment.
With the idea to identify glucose regulated genes regulated that allow a better understanding of the participation of the different signaling components, we have analyzed the expression of genes involved in the biosynthesis of plastidic isoprenoids in plants. Isoprenoids constitute the largest class of natural products and among those produced in plastids include pigments, electron carriers as well as hormones. We have confirmed that the expression of all genes from the MEP pathway is regulated by glucose at the transcriptional level. In contrast to what it has found with other glucose regulated gene, including the photosynthetic genes, the glucose and osmotic regulation over the MEP gene expression act in an opposite way. Meanwhile high glucose repress MEP transcript levels, the same genes are induced by high osmotic conditions. Thus, these genes are excellent markers to study glucose regulation in an independent form of osmotic. To gain new insights about the glucose regulation, we have analyzed the expression levels not only of the MEP transcripts, but also of their corresponding proteins. The advances of this aspect will be also presented.

Friday, March 12 – 7:30 – 8:15 PM

The Mechanism of hAT Element Transposition

Nancy Craig
Johns Hopkins University School of Medicine

T3

Transposable elements are major constituents of virtually all genomes including plants and contribute in many important ways to genome structure and function. Indeed, it was in maize that Dr. Barbara McClintock identified the first mobile element Activator (Ac). We now know that Ac is a member of a widespread superfamily of mobile elements designated the "hAT" family, members of which can be found in fungi, plants and animals including vertebrates. We are taken a biochemical approach to dissecting the transposition of Hermes, an insect member of the hAT family. We have established an in vitro system for Hermes transposition and have demonstrated that transposition occurs by a cut & paste mechanism, Interestingly, the cleavages that excise Hermes from the donor site occur via formation of DNA hairpins on the flanking donor site DNAs. A hairpining mechanism is also used in the DNA breakage events that underlie the assembly of diverse immunoglobulin genes. We are pursuing a functional and structural dissection of Hermes transposase.

Friday, March 12 – 8:30 – 9:15 PM

Phosphorus Stress Responses in Arabidopsis and Maize

Luis Herrera-Estrella
Centro de Investigacion y Estudios Avanzados del IPN

T4

Phosphorus (P) is limiting for crop yield in over 30% of the world's arable land and, by some estimates, world resources of inexpensive P may be depleted by 2050. Improvement of P acquisition and use efficiency by plants is critical for economical and social reasons. Plants have evolved a diverse array of adaptive strategies to obtain adequate P under low P availability conditions. These include alterations of root system architecture, excretion of low molecular compound and enzymes, alterations in carbon metabolism and the enhanced expression of numerous genes involved in the low P response. Our group has been using Arabidopsis and maize to study the adaptive processes to P deprivation. In Arabidopsis we have found that root

system architecture is altered by the production of an increased density of lateral roots, and longer root hairs and the alteration of root meristematic activity. Two mechanisms of P sensing have been identify in Arabidopsis: one involved in the detection of the external P concentration, which regulates the formation of lateral roots and the expression of genes involved in P excavenging and the second involved in sensing the internal P concentration that regulates primary root elongation and genes involved in P uptake. To identify genes involved in the P response, we have isolated mutants affected in the alteration of root system architecture in response to low P conditions. In maize, our Institute is currently developing a maize ESTs sequencing project using different tropical and semitropical genotypes adapted to specific environmental conditions relevant to Mexican Agriculture. The aim of the project is to sequence 100,000 ESTs derived from 5 different genotypes grown under different environmental conditions or developmental stages. To date we have produced 15 cDNA libraries of which 7 are differentially subtracted and 8 standard, and generated 25,000 ESTs. 10, 500 unigene clusters have been assembled of which over 1200 have no match to the public miaze ESTs databases. The results obtained from the analysis of the unigene clusters, in particular those derived from maize genotypes adapted to acid soils and grown under low P availability will be discussed.

SHORT TALKS

Developmental Genetics Talks

Friday, March 12 – 8:30 – 10:10 AM

Peptide-Mediated Signaling from the Egg Apparatus of Maize

Dresselhaus, Thomas; Marton, Mihaela; and Amien, Suseno
Biocenter Klein Flottbek, University of Hamburg

T5

Compared to animals, our understanding about signaling in plants is still in its infancy. In animal systems, cell-cell communication is largely mediated by signals such as steroids and peptides. Among these signaling molecules, peptides are the most commonly used, probably because of their diversity. Recently, it was shown i.e. for lower animals, that peptides secreted by the egg cell attract the sperm cells, which move along a peptide gradient. In plants, to date only a few peptides have been identified acting as signaling molecules mediating self incompatibility, cell and growth regulation, defence signaling and root nodulation.

We are interested in the regulation of cell-cell communication of the embryo sac (female gametophyte) before and after fertilization. Here, maturation of the seven celled embryo sac, guidance of the male gametophyte (pollen tube) towards the egg apparatus and post fertilization events such as cell differentiation during embryo development require very well balanced cell to cell communication systems. Secreted peptides may play key roles during these processes as signal mediators between gametic and maternal/paternal as well as embryonic cells. Using genomics/transcriptomics based approaches and differential screening methods involving cDNA libraries of egg cells, zygotes etc., we have identified several genes encoding small, secreted peptides, which are specifically expressed in the female gametophyte of maize. We will present an overview about our transcriptomics based approach, which involves the microdissection of ovular tissue and the embryo sac. The functional analysis of a few candidate genes encoding, for example, a pheromone-like Caax box peptide, peptides with homology to antifungal proteins and a peptide mediating pollen tube guidance will be presented. Functional studies include the generation of knock-outs using antisence as well as RNAi technology, GFP-localization studies and recombinant peptides.

Barren stalk1 and the Control of Lateral Meristem Initiation in Maize

Gallavotti, Andrea {1}; Zhao, Qiong {2}; Kyozuka, Junko {3}; Meeley, Robert {4}; Ritter, Matthew {1}; Doebley, John {2}; and Schmidt, Robert {1}
{1} University of California, San Diego; {2} University of Wisconsin, Madison; {3} University of Tokyo; {4} Pioneer Hi-Bred International

T6

Lateral meristems are formed throughout the life cycle of a plant creating secondary axis of growth during both vegetative and reproductive phases of development, therefore playing a major role in the determination of plant form and architecture. In maize, a unique series of lateral meristems is responsible for the formation and proper development of its unisexual inflorescences, the tassel and the ear. The maize mutant barren stalk1 is defective in lateral meristem formation during the entire life cycle, thus resulting in a mature plant having a barren stalk devoid of tillers and ears, and a tassel without branches and spikelets. These defects resemble the inflorescence phenotype of the rice lax1 mutant. Here we show that the maize

orthologue of the LAX1 gene in rice is the barren stalk1 gene. Ba1 encodes a small protein of 219 aa with a bHLH domain. Mutant analysis indicates that Ba1 is required for the initiation of all aerial lateral meristems, with the exception of those that initiate adventitious roots. The reference allele, identified in 1928, is disrupted by a Helitron insertion in the proximal promoter region. The characterization of newly identified weak alleles resulting from Mutator insertions, together with expression analysis, suggests an additional role of Ba1 in floret formation. As the differential activity of lateral meristems has contributed to the domestication of modern maize from its wild ancestor teosinte, we are investigating ba1 expression in maize lines introgressed with specific teosinte genomic regions corresponding to ba1 and teosinte branched1 (tb1) chromosomal regions. These genomic segments encompass two of the five major QTLs controlling most of the morphological differences between maize and teosinte.

Control of Phyllotaxy in Maize by ABPHYL1

Jackson, David {1}; Wang, Jing {1}; and Giulini, Anna {2}
{1} Cold Spring Harbor Laboratory; {2} University of Milan, Italy

T7

Plants initiate leaves and other organs in reproducible and characteristic geometric patterns. These patterns have been studied for decades, but how they are generated is not well understood. We are studying the ABPHYL1 (ABPH1) gene of maize. Loss of ABPH1 function causes plants to develop with opposite and decussate phyllotaxy rather than the alternating or distichous pattern that is normal for maize. We recently cloned ABPH1 using transposon tagging, and it encodes a cytokinin inducible response regulator homolog. Although this gene family has been known for several years, this is the first report of a loss of function phenotype in one of these genes. I will present data on the regulation of ABPHYL1 expression by cytokinin as well as its expression pattern, which suggests models for how this gene acts to regulate phyllotactic paterns.

Adaxial/Abaxial Specification of the Maize Leaf

Juarez, Michelle; and Timmermans, Marja
Cold Spring Harbor Laboratory

T8

Specification of adaxial(upper)/abaxial(lower) polarity is responsible for normal outgrowth and patterning of the maize leaf. Establishment of adaxial/abaxial polarity requires signals from the shoot apical meristem as well as from the leaf itself. Through molecular and genetic analyses we have identified several genes involved in setting up adaxial identity. Recessive mutations in *leafbladeless1* (*lbl1*) lead to development of radial symmetric abaxialized outgrowths suggesting lbl1 is required for proper adaxial specification. Semi-dominant mutations in *Rolledleaf1* (*Rld1*) develop adaxialized leaves or cause partial inversions of adaxial and abaxial domains in the leaf blade. *lbl1Rld1* double mutants display a mutual suppression of both the single mutant phenotypes suggesting the two mutations act in the same adaxial/abaxial pathway.

Several maize genes were cloned with high homology to two *Arabidopsis* gene families; *YABBY (YAB)* and *HOMEODOMAIN LEUCINE ZIPPER class III (HD-ZIP III),* which are known to play a role in abaxial and adaxial cell fate, respectively. In contrast to *Arabidopsis*, the maize *yab* genes are expressed in the adaxial domain of young leaf primordia. In both the *lbl1* and *Rld1* mutants yab expression is altered. This data places the maize yab genes downstream of *lbl1* and *rld1* in the adaxial/abaxial specification pathway.

We recently cloned *rld1*, which encodes the homolog of *Arabidopsis REVOLUTA*, a member of the *HD-ZIP III* family. Members of this gene family are thought to be regulated by microRNAs

(miRNA). *rld1* is expressed in the SAM and the adaxial domain of young leaf primordia. Dominant mutant alleles of *Rld1* result from mutations in the *miRNA166* complementary site and misexpress *rld1* on the abaxial side. *miRNA166* is expressed in the abaxial domain of leaf primordia, consistent with the misexpression of *rld1* in the dominant *Rld1* mutants. *miRNA166*, thus, has a role in adaxial/abaxial specification by spacial restriction of *hd-zip III*. *rld1* expression is reduced in *lbl1* mutant tissue. This together with the mutual suppressive interaction between *lbl1* and *Rld1* suggests that *lbl1* is upstream of *rld1*. The combination of the genetic and molecular analyses outlines an adaxial/abaxial specification pathway leading to proper development of the maize leaf.

Evolution of Genes Related to leafy hull sterile1 in the Grasses

Malcomber, Simon; and **Kellogg, Elizabeth**
University of Missouri, St. Louis

T9

Gene duplications are thought to provide the raw material for evolution, but there are few examples of genes whose function has diversified after duplication. We have been investigating MIKC-type MADS box genes in the grasses, and find that many of them were duplicated just prior to the origin of the family. In particular, grasses have multiple genes similar to the SEPALLATA genes of Arabidopsis. We have used a phylogenetic approach to establish orthology among the SEP-like genes, and find that the grass genes fall into several major clades: 1) a group orthologous to Arabidopsis SEP3; 2) three groups related to Arabidopsis SEP1 and 2. The latter correspond to the rice genes OsMADS1 (= LEAFY HULL STERILE1), OsMADS5, and OsMADS34. We have studied the expression of members of the OsMADS1 clade, which includes ZMM8 and ZMM14, in multiple species of grasses. The gene is strongly expressed in the spikelet meristem of all species examined. It is also expressed in the upper flower, but not the lower one the panicoid grasses, whereas it is expressed in all flowers in oats. The gene has been hypothesized to confer determinacy on the spikelet, and to restrict the number of flowers produced, but our data do not support this interpretation. As flowers mature, gene expression becomes restricted, but the precise pattern of expression varies among species. In most species examined, the gene is expressed in the lemma and palea of flowers that will form a functional gynoecium, but is not expressed in sterile or staminate flowers; maize is an exception to this pattern. In spikelets in which LHS1/OsMADS1 is expressed in a subset of the flowers, the expressing flowers have a different lemma and palea morphology than non-expressing flowers; maize and Chasmanthium latifolium (inland sea oats) are exceptions. We hypothesize that the role of OsMADS1/LHS1 has changed over evolutionary time, and that it contributes to the morphological diversity of the grasses.

knotted1 Modulates Different Hormone Pathways in Maize Compared to Dicots

Muszynski, Michael {1}; Hedden, Peter {2}; and Archibald, Rayeann {1}
{1} Pioneer Hi-Bred Intl., Inc. {2} Long Ashton Research Station, University of Bristol

T10

The maize knotted1 (kn1) homeodomain transcription factor functions to maintain the indeterminate properties of meristematic cells. Recent evidence from dicots indicates that knotted1-like homeobox (knox) misexpression phenotypes are mediated by plant hormones. Ectopic knox expression results in increased cytokinin (CK) accumulation and reduced gibberellic acid (GA) levels. In tobacco, the KNOX protein NTH15 directly inhibits transcription of the GA 20-oxidase biosynthetic gene Ntc12. In Arabidopsis, ectopic expression of KNAT1, STM or kn1 reduces accumulation of AtGA20ox1 transcript. In order to elucidate targets of

knotted1 in maize, we ectopically expressed kn1 in transgenic maize plants. Transgenic plants display characteristic knox misexpression phenotypes, including a displaced blade-sheath boundary, ectopic ligules in the blade and tissue outgrowths localized along the mid-rib. Leaf tissue from transgenic plants shows abundant accumulation of kn1 mRNA. To test if GAs mediate the ectopic kn1 phenotype, endogenous C-19 and C-20 GA levels were measured in 2-week old seedlings. Surprisingly, no differences in endogenous GA levels were detected. We also tested if GA biosynthesis genes in maize, including GA 20-oxidase, were regulated by kn1. None of the genes tested showed differential expression between the transgenic and non-transgenic sibs. We did, however, find evidence of a link to the CK pathway, as the cytokinin oxidase1 gene is differentially expressed in transgenic seedlings and exogenous CK treatment enhances the knotted phenotype. These data suggest the maize kn1 may regulate a different set of genes from those regulated by the dicot kn1 orthologs.

Biochemical Genetics Talks

Friday, March 12 – 10:50 AM – 12:25 PM

Expression of Feedback Insensitive Corn Aspartate Kinase in Corn Seed Results in an Increase of Threonine

Chang, Chun-Hsiang {1}; Ward, R. Timothy {2}; Caster, Cheryl {2}; Jung, Rudolf {1}; Falco, S. Carl {2}
{1} Pioneer, a DuPont company; {2} DuPont

T11

The aspartate-derived amino acid biosynthetic pathway leads to the synthesis of lysine, methionine, threonine and isoleucine. Activities of several key enzymes in this pathway have been altered to increase lysine and threonine. Aspartate kinase (also called aspartokinase) is the first enzyme involved in this pathway. A lysine-feedback insensitive maize aspartate kinase (mAK) was created by site-directed mutagenesis and a feedback insensitive dihydrodipicolinate synthase (dapA) was obtained from Corynebacterium. Transgenic expression of mAK either in the embryo or in the endosperm significantly increased free threonine levels and transgenic expression of deregulated dapA in corn embryos resulted in very high free lysine levels. Plants expressing the mutant enzymes were crossed. The obtained results reveal a complex relationship between the various enzymes within this pathway and these data will be discussed in the presentation.

Relevance of the Structure of the Middle Region in the Evolution of HSP100/ClpB Proteins

Nieto-Sotelo, Jorge; Luz, Maria Martinez; Zerate, Francisco; and Flores, Blanca Lidia
Institute of Biotehnology, UNAM

T12

High temperatures affect growth and development causing the accumulation of misfolded and aggregated proteins within the cell. Hsp100/ClpB chaperones facilitate the resolubilization of these protein aggregates, in conjunction with Hsp70 and Hsp40 (1). This group of proteins is found in bacteria, protozoa, fungi and plants and their expression is inducible by heat stress (2). In yeast, Arabidopsis, and maize Hsp100/ClpB proteins play a major role in the acquisition of thermotolerance, i.e., the ability to survive very high temperatures following acclimation to mild heat shock treatments (3, 4, 5). Expression of Hsp101 in maize causes a reduction in the growth rate of primary roots (5). Recently, we found that Hsp101 expression causes also a

growth reduction in stems and leaves of adult plants. In leaves, this reduction is caused at least in part by a decrease in cell proliferation activity.
Both the length and the primary and secondary structure of the middle region of Hsp100/ClpB proteins has been conserved during their evolution (6). The length of the middle region is quite different or absent in other members of the Clp family (ClpA, ClpC, ClpD) whose functions seem to be unrelated to those of ClpB proteins (2). To understand the mechanism of action of Hsp100 chaperones, we studied the structure-function relationship within the middle region. The middle region has the propensity to form a coiled-coil (6). We found that, both in yeast Hsp104 and in plant Hsp101 proteins, the middle region contains four amphipathic a-helices. Site-directed mutagenesis of the middle region of yeast Hsp104 showed that this domain is very important for function. Mutations that change the amphipathic character on helices 2 and 3, caused the loss of function of Hsp104 suggesting that these helices might play an important role in the maintenance of a coiled-coil structure. Genetic and biochemical analyses suggest that helices 2 and 3 play an important role in hexamer formation and protein stability. Molecular modeling of the region is consistent with the experimental data. Experiments are under way to obtain the structure of the middle region by means of structural analysis techniques.

A Nucleus-Encoded Sigma Factor Targeted to Both Mitochondria and Chloroplasts

Stern, David {1}; Brenchley, Jamie L. {1}; Roy-Chowdhury, Sanchita {2}; Belcher, Susan {3}; Allison, Lori A. {2}; and Barkan, Alice {3}
{1} Boyce Thompson Institute, Cornell University; {2} University of Nebraska, Lincoln; {3} University of Oregon

T13

Maize chloroplasts possess two distinct forms of RNA polymerase, termed PEP and NEP. PEP is the plastid-encoded polymerase, and resembles the bacterial enzyme possessing a catalytic core and a sigma specificity factor. NEP is the nucleus-encoded polymerase, resembles monomeric phage enzymes, and likely assembles with specificity factors whose nature is currently unknown. Mitochondria also contain a similar NEP, but are not known to possess a PEP-like enzyme. As part of a project to understand transcriptional regulation and the constituents of organellar RNA polymerases in maize organelles, we identified a nucleus-encoded sigma factor, ZmSig2B. Sig2B is encoded by one member of a 6-gene family, whose other members encode proteins targeted to the chloroplast, based on GFP transient expression assays and/or in vitro import experiments. Sig2B, however, was found to be targeted to both mitochondria and chloroplasts, raising the questions of how this dual targeting is achieved, and what function a sigma-like protein might play in the mitochondrion. We found that Sig2B has the biochemical function of a sigma factor based on reconstitution with the E. coli core enzyme, suggesting it can assemble with PEP. Sig2B did not, however, confer specificity to mitochondrial NEP, suggesting it is not the sole transcription factor for this enzyme. The regulation of Sig2B targeting was investigated using several GFP constructs and transient expression assays. Our data suggest that alternative translation start sites may be created through developmentally-regulated promoter selection, resulting in different subcellular targeting. The biological role of Sig2B is also being investigated using reverse genetics. To this end, a Mu insertional mutant was obtained from the PML collection at the Univ. Oregon. Transcriptional phenotypes in the mitochondria and chloroplasts are presently under investigation.

The Dominant White Endosperm Factor White Cap Encodes the ZmCCD1 Carotenoid Dioxygenase in a Large Multiple Copy Gene Array

Tan, Bao-Cai {1}; Liu, Lijuan {1}; Wu, Shan {1}; Lai, Jinsheng {2}; Simone, Amy {1}; and McCarty, Donald {1}

{1} University of Florida; {2} Rutgers University

T14

Carotenoids play a vital role in plant and animal development. Beta-carotene and other provitamin carotenoids that are the main dietary source of Vitamin A in humans are important nutritional constituents of grains. Efforts to enhance carotenoid content of cereal endosperm, such as creation of the Golden rice have had limited success in achieving significant dietary levels. We hypothesize that carotenoid cleavage enzymes play an important role in remodeling the carotenoids in endosperm that may limit carotenoid accumulation. An endosperm specific carotenoid mutant of maize, the White cap (Wc), provides an excellent allele to study the regulation of carotenoid turnover. Wc is a dominant mutation that conditions white crowned kernels particularly in combination with the yellow endosperm (dominant Yellow1 (y1)) background. The phenotype is dosage dependent, with three doses conditioning super white kernels. Molecular characterization revealed that Wc encodes the maize ortholog of the CCD1 (ZmCCD1) carotenoid dioxygenase. ZmCCD1 is shown to cleave a broad spectrum of carotenoids. In comparison with yellow (wc) kernels, expression of ZmCCD1 increased 40 folds in Wc kernels. ZmCCD1 is present as a single copy gene in yellow endosperm lines, whereas the Wc locus contains more than 20 copies of ZmCCD1 apparently arrayed in a large tandem repeat. Extensive cDNA sequencing identified SNP\'s that distinguish different copies in the array. The prevalence of a single SNP genotype in cDNA\'s clone from the Wc kernels suggests that one ZmCCD1 copy in the array is responsible for the dominant endosperm phenotype. This result suggests a gain of function in ZmCCD1 that causes enhanced carotenoid turnover in the Wc endosperm. The finding also suggests that blocking CCD expression in endosperm of cereals such as Golden rice may allow higher provitamin A accumulation.

The lethal leaf-spot1 *(*lls1*) Protein Which Catalyzes Chlorophyll Degradation is Localized to the Inner Chloroplast Membrane*

Tan, Bao-Cai {1}; Liu, Lijuan {1}; Wu, Shan {1}; Lai, Jinsheng {2}; Simone, Amy {1}; and McCarty, Donald {1}

{1} University of Florida; {2} Rutgers University

T15

The LLS1 protein provides an important protection against light-dependent cell death in higher plants. A functional genomics approach has now revealed that the Lls1 gene encodes pheophorbide *a* oxygenase (PaO) which catalyzes a key step in chlorophyll degradation (Pruzinski *et al,* 2003, PNAS 100: 15259-15264). We show that this gene function is conserved between monocots and dicots by direct complementation of the (*accelerated cell death 1*) mutation of *Arabidopsis* using the maize *Lls1* cDNA (Yang *et al,* 2004, Plant Mol. Biol, In Press). We provide cellular subfractionation, chloroplast uptake, and *in vivo* GFP reporter evidence to show that the LLS1 protein is localized to the inner membrane of the chloroplast in both monocots and dicots. We further show that the LLS1 protein is present in many plant species including lower plants such as moss and ferns. A bioinformatics survey revealed that *Lls1* homologues exist in algae (*Chlamydomonas*) and cyanobacteria (*Synechocystis*, *Anabaena* and *Trichodesmium*) but not in the anoxygenic bacterium *Chlorobium* (Gray *et al,* 2004, Plant Mol. Biol, In Press). Although the LLS1(PAO) protein is always present in photosynthetic tissues

expression of the *Lls1* gene increases following wounding. We interpret these findings to indicate that failure to remove pheophorbide *a* in *lls1* plants results in the accumulation of a highly phototoxic chlorophyll intermediate near chloroplast membranes. Photoactivation of this intermediate results in damage to these membranes and loss of chloroplast integrity leading to the light-dependent cell death phenotype. Interestingly, the LLS1(PAO) protein is also found in all etiolated and non-photosynthetic tissues that we tested so it appears that the ability to degrade chlorophyll is a contingent metabolic activity present in all plant cells.

Genomics Workshop Talks

Friday, March 12 – 3:30 – 5:35 PM

A Grower's Perspective on Maize Research
Davis, Gary
National Corn Grower's Association
T16

An Assembly of the Maize Genome
Schnable, Patrick; Emrich, Scott J.; Wen, Tsui-Jung; Yao Hong; Guo, Ling; Narayanan, Mahesh; Nienaber, Amy; Chou, Hui-Hsien; Aluru, Srinivas; and Ashlock, Daniel
Iowa State University
T17
Because the bulk of the maize (Zea mays L.) genome consists of repetitive sequences, sequencing efforts are being targeted to its 'gene-rich' fraction. Traditional assembly programs are inadequate for assembling such sequences because they are optimized for a uniform sampling of the genome and inherently lack the ability to differentiate highly similar paralogs. We have developed a bioinformatics pipeline for the accurate assembly of these sequences. This pipeline, which is based on innovative parallel algorithms to ensure scalability, can assemble over 850,000 genomic survey sequences (GSSs) in three hours using 64 Pentium III 1.26 GHZ processors of a commodity cluster. The resulting assembly consists of 114,000 contigs with a total size of 177 Mb (http://www.plantgenomics.iastate.edu/maize/). The average contig contains five GSSs is 1500 bp in length. Sequencing error rates in the GSSs were estimated via two independent methods. As deposited in Genbank, the GSSs have a sequencing error rate of 2.3 x10-3. More stringent trimming of these sequence reads can reduce the error rate to 3.6 x 10-4. This six-fold reduction in the sequence error rate can be achieved with only modest losses of exonic (0.8%) and intronic (4.5%) coverage. Hence, at the cost of only modest losses of gene coverage, these GSSs can be trimmed such that their quality approaches Bermuda standards. Seven ab initio programs (FGENESH, GeneMark.hmm, GENSCAN, GlimmerR, Grail, NetGene2 and SplicePredictor) were evaluated for their accuracy in predicting maize genes. FGENESH yielded the most accurate and GeneMark.hmm the second most accurate predictions from this data set. Computational and wet lab experiments established that the maize genome contains a high frequency of Nearly Identical Paralogs (NIPs).

Consortium for Maize Genomics - An Examination of Maize Gene Coverage Obtained From Shotgun Sequences Derived From Methyl-filtered and High COT Selection Libraries

Barbazuk, Brad {1}; Chan, Agnes {2}; Quackenbush, John {2}; Schubert, Karel {1}; Beachy, Roger {1}; Lakey, Nathan {3}; and Bennetzen, Jeffrey {4}

{1} Donald Danforth Plant Science Center; {2} TIGR, 9712 Medical Center Drive, Rockville, MD 20805; {3} Orion Genomics, 4041 Forest Park Avenue, St. Louis, MO 63108; {4} Department of Genetics, University of Georgia, Athens

T18

The large size of the maize genome and the expectation that upwards of 80% of the genome is represented by repetitive elements has prompted the examination of sequencing technologies expected to target gene rich regions. One objective of the Consortium for Maize Genomics is to evaluate two approaches to sequencing the maize 'genespace' (methylation filtration and high Cot selection) in order to provide the most rapid and cost-effective alternative to sequencing the whole genome.

Methyl filtration involves shotgun sequencing of the hypomethylated fraction of the corn genome, which is thought to contain most of the genes, while High Cot selection involves denaturation of total genomic DNA with heat or alkali, followed by controlled renaturation to differentiate between high copy (repetitive) and low copy (genic) fractions of the genome.

Ultimately, sequence reads from 250,000 methylation filtered clones and 250,000 high Cot clones will be obtained, and the sequences will be clustered and assembled at regular intervals. Analysis of assemblies constructed from 200,000 sequences revealed a six-fold reduction in the effective genome size, and a four-fold increase in gene hit rate compared to a non-enriched library. The results for the current assembly and an analysis of the extent of maize gene coverage and bias observed for methyl-filtered and high-Cot derived sequences within a set of curated maize genes and a set of predicted gene models will be presented.

Consortium for Maize Genomics – Assembly and Annotation of the Filtered Maize Genome

Chan, Agnes P. {1}; Pertea, Geo {1}; Cheung, Foo {1}; Lee, Yuandan {1}; Zheng, Li {1}; Barbazuk, Brad {2}; Schubert, Karel {2}; Beachy, Roger {2}; Lakey, Nathan {3}; Bennetzen, Jeffrey {4}; and Quackenbush, John {1}

{1} The Institute for Genomic Research; {2} Donald Danforth Plant Science Center; {3} Orion Genomics; {4} University of Georgia

T19

Maize is both a classical genetic model and an economically important crop; however, the structure of its genome has made sequencing maize a significant challenge. The size of the maize genome is estimated to be nearly as large as that of human, 20 times that of Arabidopsis and six times rice. Current estimates are that nearly 80% of the maize genome is comprised of highly conserved, retrotransposon and other repetitive DNA sequences with the genes distributed within unique sequence 'islands' in a 'sea' of repeats. Our objective in the Maize Genomics Consortium is to investigate approaches to sequencing the maize 'gene space' in order to provide a rapid and cost-effective alternative to sequencing the entire genome.

We have evaluated two genome filtration approaches - methylation-filtering (MF) and high Cot selection (HC) - to identify the gene representation and biases inherent in each approach. In total, we have generated nearly 1,000,000 paired-end sequence reads from 250,000 methylation-filtered clones and 250,000 high Cot clones. The sequences derived from the MF and HC libraries are assembled independently, as well as together, to evaluate the respective

coverage of the gene space provided by each approach; overall, these filtering approaches provide a four-fold enrichment of gene sequences relative to random sequences. The assembled Zea mays (AZM) sequences arising from these assemblies are annotated using a combination of homology searches and ab initio gene prediction programs. To discriminate repetitive sequences during subsequent analyses, we have also developed a maize repeat database that contains both known repeat sequences and novel repeats discovered using the RECON repeat-finding algorithm. Results of these analyses are presented along with summary information at the TIGR Maize database website (http://www.tigr.org/tdb/tgi/maize). A detailed overview of the project and the results derived from our analysis will be presented.

High Resolution Physical Mapping of the Maize Genome and Sequencing a Part Thereof

Bharti, Arvind K. {1}; Wei, Fusheng {2}; Butler, Ed {2}; Yu, Yeisoo {2}; Goicoechea, Jose L. {2}; Kim, HyeRan {2}; Fuks, Galina {2}; Nelson, Will {3}; Hatfield, Jamie {3}; Gundlach, Heidrum {4}; Karlowski, Wojciech M. {4}; Raymond, Christina {5}; Towey, Sarah {5}; Jaffe, David {5}; Nusbaum, Chad {5}; Birren, Bruce {5}; Mayer, Klaus {4}; Soderlund, Cari {3}; Wing, Rod A. {2}; and **Messing, Joachim** {1}

{1} The Plant Genome Initiative at Rutgers (PGIR), Waksman Institute, Rutgers; {2} Arizona Genomics Institute (AGI), University of Arizona; {3} Arizona Genomics Computational Laboratory (AGCoL), University of Arizona; {4} Munich Information Center for Protein Sequences (MIPS), Institut für Bioinformatik; {5} MIT/Broad Institute Center for Genome Research (MITCGR)

T20

Due to its economic significance, maize (>2 Gb) is likely to be the next cereal to be sequenced after rice (0.4 Gb). Sequencing the maize genome will present a new challenge not only because it is 5-times larger but also because it contains many gene families, tandemly arrayed and nested repeat sequences. To establish a framework to sequence maize, we are developing a high information-content fingerprinting (HICF)/BAC sequence tagged connector (STC)-based physical map. This map will be fully integrated with the genetic map and is complementary to previous work by the NSF funded Maize Mapping Project (www.genome.arizona.edu/fpc/maize). MMP generated a genetically anchored BAC-based physical map by fingerprinting three deep coverage BAC libraries (*Hin*dIII, *Eco*RI, and *Mbo*I) using a lower resolution “agarose” method. The HICF method builds upon the MMP physical map by generating HICF fingerprints of the identical libraries, which allows for a more precise determination of a minimum tiling path of BAC clones across the entire genome. As of now, >403,000 successful fingerprints (25x) and ~400,000 BAC end sequences (>250 Mb, more than 10% of the genome) have been generated. Preliminary investigation suggests that many HICF mega-contigs are likely to join two or more agarose-based contigs. The current HICF fpc build has resulted in 2,947 contigs, which is expected to further reduce after manual editing. Additional contigs are expected to be anchored based on alignments of STCs to the rice genome. To evaluate the MTP generated by the HICF/agarose physical map, 56 BACs are being sequenced from a ~5 Mb mega-contig, in addition to 100 randomly selected BACs (>10 Mb). All sequence information generated from this project, from both BAC ends and whole BACs, is being annotated, which will provide invaluable data on the sequence organization of the maize genome.

Techniques for Finishing and the Assembly of Gene-Enriched Shotgun Sequence Data into a Linked Archipelago of Beautiful Gene Islands, Beaches and All

Bennetzen, Jeff {1}; Yuan, Y {2}; Emberton, J {2}; Ma, J {1}; Estep, M {1}; Luo, M {3}; Wing, R {3}; and Soderlund, C {3}
{1} University of Georgia; {2} Purdue University; {3} University of Arizona

T21

Gene-enrichment sequences, including Methylation Filtering (MF) and High Cot selection (HC) will provide the sequences for most or all maize genes within the coming months. At a low sequence redundancy, some genes may be missed and many genes will only be partially sequenced. Cost effective and comprehensive techniques are needed to complete the sequences of all of these genes and link the sequenced gene islands to both the genetic and physical maps of the maize genome. We have developed methylation spanning linker library (MSLL) technology as an approach to locate all gene islands relative to each other, and thus to any maize map. We also are using hypermethylated partial restriction (HMPR) libraries as a technique that can efficiently finish gene-enriched shotgun draft sequences. These and other similar approaches will be discussed. The current status of MSLL and HMPR studies on maize will also be described.

Maize Genome Sequencing By Methylation Filtration

Rabinowicz, Pablo {1}; Palmer, Lance {1}; O'Shaughnessy, Andrew {1}; Balija, Vivekanand {1}; Nascimento, Lidia {1}; Hemann, Michael {1}; Lowe, Scott {1}; May, Bruce {1}; Vollbrecht, Erik {2}; Lucito, Rob {1}; McCombie, W. Richard {1}; and Martienssen, Rob {1}
{1} Cold Spring Harbor Laboratory; {2} Iowa State University

T22

Gene enrichment strategies offer an attractive alternative to sequencing large and repetitive genomes such as that of maize. We have generated 100,000 under-methylated or "Methylation Filtration" (MF) sequence reads from maize and analyzed them together with 500,000 MF reads submitted to GenBank by the Maize Genomics Consortium. Comparing MF, EST and Rescue Mu sequences with the rice genome reveals that MF results in more comprehensive representation of maize genes than do the other two sequencing approaches. About 7% of maize repetitive DNA is unmethylated and thus, selected in MF libraries. Unmethylated repeats are mostly decayed or potentially active, unmethylated transposons. MF results in gene enrichment in most plant species but not in mammals. Using McrPCR, a new approach to study DNA methylation, we showed that most exons are unmethylated in plants while the majority of animal exons are methylated.

Cytogenetic and Transposon Talks

Saturday, March 13 – 8:30 – 10:10 AM

Somatic Karyotype Analysis in Maize

Kato, Akio; Lamb, Jonathan; and **Birchler, James**
University of Missouri

T23

Distinguishing each of the ten maize chromosomes has been possible at the pachytene stage of meiosis for many decades, but making this distinction in somatic root tip cells has been difficult because the chromatin is highly condensed. Discrimination of each chromosome is further complicated by polymorphism for heterochromatic knob regions, which change the chromosome

arm ratios in different varieties. Repetitive DNA sequences of maize were used to develop a fluorescent in situ hybridizaton (FISH) method to identify each of the ten chromosomes in somatic cell spreads. Various types of centromeric repeats, knob heterochromatin, subtelomeric sequences, microsatellite clusters and the ribosomal RNA gene arrays were utilized via FISH to provide each chromosome with a distinctive pattern of hybridization. Despite the fact that significant variation exists for many of these types of repetitive sequence clusters, each of the ten chromosomes of maize can be distinguished in root tip spreads in all inbred lines (15+) examined to date. Identification of each of the ten chromosomes is also possible at pachynema, diplonema and metaphase of meiosis. Direct labeling of the probes with fluorescent nucleotides rather than antibody amplification results in low background with high signal. This procedure has been used in our laboratory to detect chromosomal aberrations in somatic cells, to study chromosomal behavior, to determine specific chromosomes present in aneuploids and to localize large transgenes to chromosomal regions. The potential exists to combine the chromosomal identification kit with sensitive detection of individual genes or transgenes to place them on the somatic karyotype.

Eludicating the Cohesion Protein Network by Analysis of Maize Mutants

Hamant, Olivier {1}; Golubovskaya, Inna N. {1}; Braun, David {2}; Pawlowski, Wojtek {1}; and Cande, W. Zacheus {1}

{1} University of California, Berkeley; {2} Pennsylvania State University

T24

Characterization of meiotic mutants has shown that sister chromatid cohesion (SSC) is required for establishing a proper meiotic chromosome structure and behavior during early meiotic prophase. In the afd1-1 mutant, equational instead of reductional segregation of sister chromatids occurs during the first meiotic division due to a failure in both arm and centomere SSC. In addition, chromatin is not properly organized during meiotic prophase and the early prophase I stages, leptotene, zygotene, and pachytene, are bypassed in afd1-1. Neither homologous pairing nor synapsis take place, and the synaptonemal complex does not form. We found a novel afd1 allele, afd1-2, tagged with a Mu1 transposon in a collaborative effort with D. Braun. The afd1-2 meiotic phenotype is very similar to the phenotype of the original afd1-1 mutant. Molecular analyses indicated that a Mu1 insertion caused a ca. 100 kb deletion encompassing a homolog of the yeast meiotic specific cohesin REC8. The phenotypes of the afd1 mutants are close to the phenotypes of the rec8 mutants in yeast, which are defective in SSC and meiotic recombination. Sequencing the afd1-1 allele revealed a point mutation introducing a premature stop codon, thus confirming that AFD1 is a maize REC8 homolog. Three other genes with similarities to AFD1 have been identified in maize, but based on sequence alignments, they are closer related to RAD21, the mitotic homolog of REC8, than to REC8 itself. The expression profile of AFD1 and the three ZmRAD21 in wild-type and in several mutants impaired in SCC during meiosis is being investigated. In order to find mutants that uncouple the two different functions of AFD1, SSC and chromosome structure, 13 new mutant alleles have been obtained (Pionneer) and are currently being characterized. In a different screen, we found a novel maize meiotic mutant, mtm99-31, which, in contrast to afd1, displays a normal first meiotic division with completely homologous synapsis and reductional chromosome segregation. However, sister chromatid centromere regions precociously separate from each other before second meiotic division, resulting in random segregation of chromosomes. This phenotype is similar to yeast and Drosophila mutants defective in proteins that protect REC8 from cleavage before the second meiotic division. We are currently doing molecular and genetic

analysis on these proteins in the WT and mtm99-31 to determine the role of the cohesins network in controlling SSC and chromosome structure during meiosis.

Cross Incompatibility Between Maize and Annual Mexican Teosintes

Kermicle, Jerry {1}; and Evans, Matthew {2}
{1} University of Wisconsin, Madison, WI; {2} Carnegie Institute, Stanford, CA
T25

As female parent some teosintes are unreceptive to maize pollen. By treating failure of seed set as a heritable trait we incorporated the genes involved into Midwestern dent lines. One group of derived lines carries the popcorn gametophyte factor Ga1-s. However, the Mexican maize landraces sympatric to these teosintes proved to be Ga1-m Ga1-m. Although plants of this genotype lack the silk barrier, their pollen can fertilize Ga1-s Ga1-s, thereby negating Ga1-s as a barrier to crossing. A second group of derived stocks carry teosinte crossing barrier-1. The Tcb1-s pistil barrier is dominant and, like Ga1-s, pollen competence is determined gametophytically. Tcb1-s was present in one of the four spp. parviglumis populations characterized, was not detected in two wild/ruderal spp. mexicana populations and was polymorphic in a third. It was prevalent if not fixed in the four weedy populations of mexicana populations tested. These four are representative of those present in the Valley of Mexico and Central Plateau regions where teosinte tends to mimic the local maize and where hybrids are relatively infrequent even though flowering times overlap extensively. Tcb1-s was not found in the sympatric maize populations. Thus pollen-pistil incompatibility conferred by Tcb1 may serve to prevent weedy teosintes from being fertilized by maize.

A New Twist on DNA Repair: Characterization of the Maize Mre11 Gene(s)

Altun, Cagla {1}; Meeley, Bob {2}; Mahajan, Pramod {2}; and Weil, Clifford {1}
{1} Purdue University; {2} Pioneer Hi-Bred
T26

During excision, Class II transposons, such as Ac, break both DNA strands at the donor site. How these breaks are repaired is not completely clear and, although host proteins are involved, what proteins participate in the transposition complex remains unknown. Data from yeast cells and from Arabidopsis suggest that factors in the nonhomologous end-joining pathway (such as mre11, rad50, ku70 and ku80), also play roles in repairing Ac excision sites. In addition, the PSO2 gene, typically part of interstrand cross-link repair, appears to be involved in positioning Ac transposase at the element ends.

We have characterized the maize mre11 gene, one of the only genes known to be involved in two major, independent, double strand break repair mechanisms in eukaryotes, as well as repair of Ac excision sites. Data suggest that its endonuclease activity might have a role in the opening of DNA hairpins that form during transposition.

In maize, the mre11 transcription unit is 5.5 kb, encoding an mRNA that has at least two alternatively spliced 3' ends in leaf tissue. One of these RNAs would encode a protein with similarity to other eukaryotic Mre11 proteins along its entire length, while the other RNA encodes a truncated protein lacking the C-terminal DNA binding domain. We are currently characterizing three different mre11::Mu alleles obtained from the Pioneer Hi-Bred TUSC collection, all with insertions in the promoter region. Arabidopsis mre11 mutations (T-DNA insertions as well as a truncation identified by TILLING) produce smaller plants and partial to complete sterility compared to nonmutant sibs. Preliminary data in maize indicate similar developmental abnormalities associated with the mre11::Mu alleles. We will present results characterizing the effects of AtMre11 mutations on repair of Ac excision sites.

Using a reverse genetics approach, we have also identified a second, putative mre11 homolog in maize, mre11B, located in the 19 kD zein cluster. The predicted protein, 61% identical to maize mre11A, shows strong similarity to the N-terminal region that encodes the Mre11 nuclease domain. It ends at approximately the same position as the prokaryotic mre11 homolog sbcD, although it is not similar to sbcD and no organellar targeting sequence is apparent. mre11B is not represented in existing EST databases, suggesting that, if it is expressed, it may be highly tissue-specific. TUSC screens have produced two Mu insertion alleles predicted to affect mre11B, one disrupting an important predicted exon, and we are characterizing these alleles further.

Epigenetic Silencing of MuDR/Mu Transposon

Ono, Akemi; and Walbot, Virginia
Stanford University

T27

MuDR programs transposition of diverse Mu transposons. MuDR contains two genes: mudrA and mudrB. mudrA encodes MURA transposase that binds to a 32 bp motif within the Mu terminal inverted repeats (TIRs). mudrB has limited homology to one domain in the phage Mu B protein. Both MuDR transcripts are abundant, and MURA and MURB proteins are ubiquitously present in somatic tissue. Stocastically during development in some individuals, unlinked MuDR elements undergo coordinate epigenetic silencing measured as a decrease in MuDR transcripts, no somatic excision transposition, and methylation of Mu TIRs. To address the mechanisms of MuDR silencing, transgenic corn expressing cauliflower mosaic virus 35S promoter:mudrB (35S:mudrB) was crossed to a multi-copy MuDR line; native mudrB and 35S:mudrB transcripts were monitored by RT-PCR in active and sister individuals in which silencing occurred. During somatic silencing, mudrB and 35S:mudrB transcripts decreased in parallel and progressively in successively later somatic organs. In the next generation, however, although mudrB expression was extinguished in fully silenced lines, 35S:mudrB levels were restored to normal levels. As a reciprocal experiment, transgenic corn expressing a MuDR TIR promoter:luciferase (TIR:LUC) gene was crossed to a multi-copy MuDR line; native mudrB and TIR:LUC transcripts monitored. In contrast to the decrease in 35S:mudrB during somatic silencing, TIR:LUC transcripts were equally abundant in active, silencing, and silenced individuals. These results indicate that initiation of MuDR silencing in dispersed elements depends on the coding region of a MuDR gene such as mudrB and likely involves mRNA destruction through post-transcriptional gene silencing. Imposition of a heritable silenced state, presumably through transcriptional gene silencing, additionally requires promoter homology in cis to a region of transcript homology. In addition to the transcriptional levels, we are analysing the distribution of the methylation of MuDR during silencing and in fully silenced lines using restriction nuclease digestion and bisulfite sequencing. Research was supported by a grant from the National Institutes of Health.

QTL, Epigenetic, and Cell Biology Talks

Saturday, March 13 – 10:40 AM – 12:20 PM

Allelic Variation of Gene Expression in Maize Hybrids

Guo, Mei {1}; Rupe, Mary {1}; Zinselmeier, Christopher {1}; Habben, Jeffrey {1}; Bowen, Benjamin; and Smith, Oscar {1}
{1} Pioneer Hi-Bred International, Inc.

T28

Naturally occurring allelic diversity in plants is an important component of genetic variation underlying phenotypic variation. Parental allelic differences give rise to heterozygosity in a hybrid genome, which presumably contributes to the genetic basis of heterosis or hybrid vigor. Virtually nothing is known about the expression level of the parental alleles in a hybrid. We examined parental transcript accumulation in maize hybrids by using allele-specific RT-PCR analysis. Among 15 genes analyzed, 11 showed differences at the RNA level, ranging from unequal expression of the two alleles (bi-allelic) to expression of a single allele (mono-allelic). Maternal or paternal transmission had little effect on the allele-specific transcript ratio of nearly all genes analyzed, suggesting that parent-of-origin effect was minimal. We analyzed the allelic difference in genetically contrasting hybrids, and hybrids under high density and drought stress. While a genetically improved modern hybrid expressed both alleles, a less improved 'old' hybrid frequently showed mono-allelic expression. This result implies the role of regulatory allelic variants in the improvement of modern hybrids. Furthermore, the two alleles in the hybrid responded differentially to abiotic stresses. The results of allele-specific regulation in different tissues, in responding to environment and stress suggest an unequivalent function of the parental alleles in the hybrid. The advantage of bi-allelic expression in hybrids over mono-allelic expression in inbreds may have an impact on heterosis. The extensive allelic expression variation among naturally occurring alleles suggests its significant role in affecting quantitative trait variation.

Advanced Backcross Analysis of Maize / Zea diploperennis: Identification and Verification of Novel QTL with Agronomic Importance in Hybrid Maize

Harjes, Carlos E. {1}; Smith, Margaret E. {1}; McCouch, Susan R. {1}; and Tadmor, Yaakov {2}
{1} Cornell University; {2} Agricultural Research Organization, Volcani Center

T29

The advanced backcross (AB) molecular marker assisted breeding approach was used with a maize / Zea diploperennis (Zd) backcross two (BC2) population to determine the potential of this strategy to exploit 'wild' genetic diversity for hybrid maize improvement, and characterize Zd as a donor. In our maize wide cross-derived backcross the value of this method was dependent on the trait of interest, existence and magnitude desirable novel alleles in the donor, and the QTL mapping power conditioned by population size and skewing.

QTL analysis, based on the phenotypic evaluation of 194 BC2 testcross progeny at four locations, revealed the expected large number of teosinte alleles with a negative impact on yield. However, the opposite was observed for grain quality, where all QTL detected had positive effects. Over the 23 traits evaluated 137 putative QTL were identified in this study. Of these QTL more than 50% did not co-localize to bins previously associated with maize QTL for the respective traits indicating a high frequency of novel loci.

The major advantage of the AB method in manipulation of QTL for crop improvement is that detection of QTL and rapid generation of nearly isogenic lines is conducted in the target elite genetic background. Two QTL selected for increased grain protein and oil content, and two for prolificacy were introgressed and verified in our recurrent parent, the target elite inbred, confirming the utility of the AB QTL approach for these traits. Mean effects confirmed for these QTL ranged from 7% to 10% increased grain protein and oil concentration, and 8% to 12% increased prolificacy. On the other hand, no gains were attained for grain yield, suggesting this approach is not appropriate for this trait when using a wild donor in contrast to earlier AB studies in self-pollinated crops. This may in part be conditioned by the tailoring of elite inbred lines for hybrid performance, and the requisite BC2 testcross phenotypic evaluations used in this study,

in addition to a reduced frequency of favorable donor alleles for yield. In maize, gene flow from wild ancestors is likely to have occurred over a long period of domestication, providing ample opportunity for selection of useful Zea alleles for yield, however selection for loci improving grain protein and oil content is unlikely to have occurred.

RNA Silencing of an Endogenous Gene in Maize

Della Vedova, Chris; Cone, Karen; and Birchler, James
University of Missouri

T30

RNA silencing refers to the targeted degradation of an mRNA by a homologous short interfering RNA (siRNA) and an associated protein complex. Described initially in transgenic systems, there is an accumulating body of evidence that RNA silencing is crucial for many aspects of plant growth and development including transposable element silencing, centromere function and developmental transitions.

We describe the mechanism of inhibition by the C2-Idf allele, a dominant allele of colorless2, which is required for the accumulation of anthocyanin pigments. The C2-Idf allele appears to initiate RNA silencing of the wild-type C2 allele when in a heterozygous state. In C2-Idf/C2 plants, accumulation of c2 mRNA is significantly reduced, but the rate of transcription is only marginally changed compared to wild-type plants.

Several hallmarks of RNA silencing are found in C2-Idf/C2 plants. C2-Idf has a more complex DNA structure than wild-type including multiple copies of promoter and 3â•· UTR homologous sequence. The C2-Idf allele is hypermethylated relative to wild-type C2. In both heterozygous C2-Idf/C2 and homozygous C2-Idf/C2-Idf plants, c2 homologous siRNAs are present indicating that C2-Idf is able to induce the RNA mediated silencing not only of the wild-type allele, but also of itself.

We examined the effect of plant viruses that encode suppressors of RNA silencing. Both Maize Dwarf Mosaic Virus (MDMV) and Maize Necrotic Streak Virus (MNeSV) encode genes that are effective inhibitors of RNA silencing. When infected with these viruses, C2-Idf/C2 plants exhibit an increase in anthocyanin and c2 mRNA levels, consistent with a release of silencing. Interestingly, in both cases there is an increase in levels of siRNAs.

Finally, C2-Idf silencing appears to be tissue specific. The allele is an effective inhibitor of wild-type C2 in adult tissues. However, in the developing embryo, the mutant allele acts as a recessive loss-of function mutation. No c2 homologous siRNAs are found in C2-Idf/C2 or C2-Idf/C2-Idf embryos. This indicates that in maize, RNA silencing may be repressed in some tissues, perhaps for developmental reasons.

ZmMEG1-1 is an Endosperm Transfer Cell-Specific Gene with a Maternal Parent-of-Origin Pattern of Expression

Gutierrez-Marcos, Jose F. {1}; Costa, Liliana M. {1}; Biderre-Petit, Corinne {2}; Khbaya, Bouchaib {2}; O'Sullivan, Donal {3}; Wormald, Mark {4}; Perez Pascual {2}; and Dickinson, Hugh G. {1}

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T31

Growth of the maize endosperm is tightly regulated by maternal zygotic and sporophytic genes, some of which are subject to a parent-of-origin effect. We show here a novel gene, *Zea mays*

Maternally-Expressed Gene1-1 (*ZmMEG1-1*), which belongs to a large gene family and exhibits a maternal parent-of-origin pattern of expression during early endosperm development only. At later stages of development, *ZmMEG1-1* is expressed from both parental alleles. We generated a stable reporter fusion containing the ZmMEG1-1 promoter, which also displays a similar pattern of expression. *ZmMEG1-1* is exclusively expressed in the basal transfer region of the endosperm. Transactivation experiments show that ZmMEG1-1 promoter is transactivated by ZmMRP1, a transcriptional activator of other genes expressed in the transfer region. Further, we show that the putatively glycosylated protein is localised to the labyrinthine ingrowths of the transfer cell walls.

Protein Kinase CK2 Modulates Developmental Functions of the Abscisic Acid Responsive Protein RAB17 From Maize

Pages, Montserrat; Riera, Marta; Figueras, Merce; Lopez, Cristina; and Goday, Adela
Departament de Genetica Molecular, IBMB

T32

The maize Abscisic acid (ABA) responsive protein Rab17 is a highly phosphorylated LEA (late embryogenesis abundant) protein involved in plant responses to stress. In this study, we provide evidence of the importance of Rab17 phosphorylation by protein kinase CK2 in growth related processes under stress conditions. Live cell fluorescence imaging of both CK2 and Rab17 indicate that the intracellular dynamics of Rab17 is regulated by CK2 phosphorylation. We found both CK2 subunits and Rab17 distributed over the cytoplasm and nucleus. By contrast, catalytic CK2 subunits and a Rab17 mutant protein (mRab17) which is not substrate for CK2 phosphorylation remain accumulated in the nucleoli. Dual colour image shows that the CK2 holoenzyme accumulates mainly in the nucleus. We also show the specific interaction of Rab17 with the CK2 regulatory subunits CK2-1 and CK2-3 and that these interactions are not dependent on the phosphorylation state of Rab17. Transgenic plants overexpressing Rab17 but not mRab17, arrest the process of seed germination under osmotic stress conditions. The retention of unphosphorylated Rab17 in the nucleolus may be a means of preventing from functioning. Thus, the role of Rab17 in growth processes is mediated through its phosphorylation by the protein kinase CK2.

Genetic Diversity Workshop Talks

Saturday, March 13 – 10:40 AM – 12:20 PM

Variation in Latin American Maize

Goodman, Major M.
North Carolina State University

T33

While Mexico has the greatest genetic variation in maize, there is considerable variation elsewhere. The US Northern Flints and the Coroicos of the Amazon basin are two of the more extreme types. There is considerable variation among the popcorns of Latin America, and a wide extreme of adaptation in the Andes, with Confite Puneño grown at elevations in excess of 10,000 feet. Some accessions of Pira from Colombia and Venezuela have very fragile cob rachises, and Cuzco Gigante from Peru and Jala from Mexico have extremely large kernels and ears, respectively. The Montañas from Colombia and Ecuador are extremely tall and late, even under short days. Compared to US maize, the variation found in most Latin American countries is extreme.

Maize Genetic Diversity

Smith, Stephen
Pioneer Hi-Bred International

T34

Genetic diversity has played a crucial role in allowing improved productivity of crop plants. Genetic diversity will assume an increasingly important role in the future to allow for the development of crops that continue to be resistant to pests, diseases, and abiotic stresses and which can contribute to a more healthy environment. Biotechnologies have an important role, not only in providing novel genetics, but also in helping to understand and to better manage crop genetic diversity. However, at the end of the day, there must be an available repertoire of useful genetic diversity to sustain advances in agricultural productivity. Patterns of genetic diversity change as farmers adapt landraces and as commercial forces change traditional roles of farmers as conservators. Conscious efforts are therefore required to conserve and to better utilize genetic resource diversity. Public/private collaborations can play an important role in providing improved stewardship of genetic resources and to better understand the socio-economic impacts of intellectual property protection and the use on-farms of new varieties. Use of genetic diversity must be combined with stewardship of those resources to allow for sustainable development of agriculture in the service of health, economic and political security.

A Multilocus Investigation of the Domestication Process in Maize

Tenaillon, Maud
Station de Genetique Vegetale, Ferme du Moulon, France

T35

The domestication of maize (*Zea mays* ssp. *mays*) from its wild ancestor (*Zea mays* ssp. *parviglumis*) led to a loss of genetic diversity both through a population bottleneck and through directional selection at agronomically important genes. In order to discriminate between those effects and to investigate the nature of the domestication bottleneck, we analysed nucleotide diversity data from twelve chromosome 1 loci in *parviglumis*. We found an average loss of nucleotide diversity of 38% across genes, but this average was skewed downward by four putatively selected loci (*tb1*, *d8*, *ts2* and *zagl1*). To better understand the domestication process, we used the coalescent with recombination to simulate bottlenecks under various scenarios. For each locus, we determine the likelihood of the observed data using goodness-of-fit statistic. Our results show that the four putatively selected loci had significantly different likelihood optimums than the neutral loci. Overall, the best-fitting models had a bottleneck in which the population size and the bottleneck duration had a ratio of ~4- to ~5. However, this range did vary with the summary statistic used to assess the fit of simulations to data. In this context, Tajima's *D* performed poorly, suggesting that *parviglumis* has a frequency spectrum that is significantly skewed towards low frequency variants.

Accessing Useful Diversity from the CIMMYT Maize Genetic Resources Collection

Warburton, Marilyn
CIMMYT

T36

The International Maize and Wheat Improvement Center's (CIMMYT) Genetic Resource Center contains over 23,000 maize landrace accessions, from which breeders have created elite breeding materials including pools, populations, and inbred lines. However, many breeders have been reluctant to use unadapted germplasm in their breeding programs (either tropical materials in temperate maize breeding programs, or germplasm bank and unadapted races in

tropical maize breeding programs). Attempts to expand diversity in breeding materials have increased recently, either by improving unadapted germplasm to expand the genetic base of maize breeding populations en masse, or by extracting new alleles at targeted genes for introduction via backcrossing. Past molecular marker diversity studies of CIMMYT maize germplasm reveal no clear picture of stratified diversity in maize races, populations, or lines, except at a few loci linked to selected traits. This is in contrast to studies done on temperate maize germplasm and some tropical maize germplasm from smaller breeding programs, which frequently show clear and unambiguous groups. Very high allelic variation was found in CIMMYT germplasm using SSR markers. Many of these alleles were found at low frequencies and were unique to the populations in which they were found. This data shows the promise of finding useful variants of known genes in CIMMYT germplasm, if we can identify them. The Challenge Program entitled 'Unlocking Genetic Diversity for the Resource Poor' proposes to find and incorporate new alleles of genes of interest from elite maize breeding materials and maize genetic resources. The strategy to be employed consists of characterization of the structural diversity of up to 1000 inbred lines, individuals from a population, or entire populations with 50 markers (SSRs or SNPs) to better predict shared pedigree or geographical origin and to find population structures that influence the analysis of functional characterization, such as associations between markers and phenotypes. This information will be used to narrow down the original set to 100 individuals for functional characterization, which will be done phenotypically, and for diversity at genes of interest (via gene expression and/or sequencing). Genes of interest are those already identified in previous structural and functional genomics studies, and will initially focus on drought tolerance and nutritional quality. Following identification, new allelic variants will be tested and incorporated in new elite cultivars.

Exploring Maize Genetic Diversity to Understand Light Response Pathways

Costich, Denise E.; and Brutnell, Thomas P.
Boyce Thompson Institute for Plant Research

T37

Phytochromes control a number of physiological processes throughout the life cycle of the plant including agronomically important traits such as tillering, plant stature and flowering time. To investigate phytochrome response pathways in maize, we examined the effects of multiple light environments on the inhibition of mesocotyl elongation. In the field, the elongation of the mesocotyl moves the shoot apex through the soil. Near the surface, light inhibits mesocotyl elongation and photomorphogenic development proceeds. Under defined light and temperature conditions, mesocotyl length provides a quantitative measure of light response. Thus, mesocotyl length was used to survey light response in a diverse maize germplasm collection consisting of over 100 semitropical, stiff stalk and non-stiff stalk inbreds. Measurements of mesocotyls were taken after 10 days of growth in the dark or under continuous red, far-red, blue or white light conditions. Our analysis indicates that North American Corn Belt stiff stalk and non-stiff stalk inbreds are less sensitive to all wavelengths of light compared to semitropical inbreds. These results suggest that artificial selection has acted to attenuate light response pathways as varieties were developed for more temperate regions (Markelz et al, 2003). Furthermore, mesocotyl length was highly correlated among light treatments. That is, mesocotyls that were long under one light condition were long under all light conditions. This result suggests that the variation in light response is not due to variation in phytochrome photoreceptors, but is likely attributable to genes that control both red/far-red and blue light response pathways. Data will be presented detailing our current efforts to identify candidate genes and associated polymorphisms that underlie mesocotyl length variation in maize.

Museum Tour

Saturday, March 13 – 6:00 – 9:30 PM (at museum)

A Story of Maize: Archaeological Evidence from Mexico
Benz, Bruce
Texas Wesleyan University
T38
The earliest archaeological evidence of maize cultivation comes from Mexican cave deposits. Archaeological deposits in Guila Naquitz, a rockshelter located east of Oaxaca City, Oaxaca, contain the earliest maize inflorescence fragments known. These 6000 year old fragments suggest that maize is a domesticated form of teosinte, a wild grass occurring in Mexico and Central America, that was planted and harvested by Middle Archaic foragers. Corn cob fragments from archaeological sediments in San Marcos cave, located south of Tehuacan, Puebla suggest early Late Archaic collectors enjoyed significant morphological improvements over the earliest maize from Guila Naquitz that enhanced harvesting. The earliest remains of maize from Coxcatlan Cave in the south of the Tehuacan valley, indicate humans collectors were devoting effort to improving harvesting characteristics, increasing grain size and ear productivity. Changes observed during the Late Archaic in northeastern Mexico document significant increases in yield comparable to those seen during the Late Formative Period in the Gulf Coast 1500 years later. Maize diffused widely during the Late Archaic Period but the greatest post-Archaic period changes in maize's productivity are noted during the Middle to Late Formative Period or Neolithic which was a time of significant social and political change Yield continues to increase through out the Classic and PostClassic periods when regions of Mesoamerica were dominated by a few, dominant urban centers. The dissolution of these urban centers and demographic and political reorganization of was accompanied by additional increases in yield. Following the Spanish conquest yields increased yet again though not to the extent that was seen during prior time periods. Regional populations of maize have come to reflect a common physiological and agronomic character that stem from biogeographical constraints and past historic and prehistoric cultural practices.

Bioinformatics and Genomics Talks

Sunday, March 14 – 9:00 – 10:40 AM

Global Picture of Linkage Disequilibrium Assessed on Maize Unigene Set in Maize Inbred Lines
Vroh, Bi Irie {1} {2}; McMullen, Michael D. {1} {3}; Schroeder, Steve {1}; Sanchez-Villeda, Hector {1}; Gardiner, Jack {1}; Polacco, Mary {1} {3}; Fang, Zhiwei {1}; and Coe, Edward H., Jr. {1} {3}
{1} University of Missouri, Columbia; {2} Cornell University; {3} USDA-ARS
T39
Linkage disequilibrium (LD), the non-random association of alleles at different loci is of interest for association mapping and for understanding genome structure. LD exploits historical recombinations in natural populations, therefore using many more informative meioses than a traditional linkage mapping population. Using LD mapping or a joint linkage and LD mapping

can accelerate the identification and fine mapping of QTL underlying many important agronomic traits. Despite its importance and utility, analysis of LD remains scarce in maize. The few studies of LD in maize have reported various rates of LD decay using a few genes. Among the published works on LD in maize, the structure of LD along the 10 chromosomes maize is lacking. So far, only Tenaillon et al. 2001 (PNAS, 98: 9161-9166) have investigated the variation of LD along maize chromosome 1 using 21 loci.

Simultaneous Prediction of microRNAs and Their Target mRNAs Acting By Translational Repression

Arteaga-Vazquez, Mario Alberto; Perez, Juan Caballero; Martinez de la Vega, Octavio; and **Vielle-Calzada, Jean Philippe**
CINVESTAV-IPN
T40

MicroRNAs (miRNAs) are short (21 to 25 nucleotides) regulatory antisense RNAs that repress the translation of their target mRNA without causing RNA degradation. The activity of a miRNA depends mainly on how precisely its molecular structure anneals to the target sequence. In animals, the few miRNAs for which a target has been discovered repress protein translation by imprecisely annealing at several locations in the 3' UTR of the corresponding mRNA. The presence of nucleotide mismatches between the two single-stranded RNAs has been shown to be essential for avoiding mRNA degradation. In plants, nearly 100 small RNAs have been identified by molecular or bioinformatic approaches, but most cause degradation of their target mRNA by perfectly annealing to their sequence at a single location in either the regulatory or the coding region. Several small RNAs have been shown to act as small interfering RNAs (siRNA) through the RNAi-pathway during mRNA degradation, and no miRNAs repressing translation by imprecise annealing in the 3' UTR have been discovered in plants. We have developed a bioinformatic approach that simultaneously identifies candidate microRNAs and their potential 3' UTR imperfect target sites in Arabidopsis thaliana. We designed a data base containing all the intergenic genomic regions and generated a program that compares their sequence to a collection of 3' UTR sequences belonging to mRNAs publically available. By allowing non-cannonic G:T pairing, the program identifies intergenic oligonucleotides (21 to 22 nt in length) that imperfectly match at least two independent sequences of a single 3' UTR. Segments of the intergenic regions containing the mature microRNA candidates are subsequently analysed to determine predicted secondary structures using MFOLD (Zucker, 1989. Science 244:48-52). Based on the systematic analysis of 3' UTRs from 13,000 mRNAs, we identified 10 microRNA candidates and their potential targets. Interestingly, our program predicted that 5 of the candidates have potential targets in the 3' UTR of RNA-binding proteins involved in pre-mRNA processing and transport. We have validated our bioinformatic analysis by determining that at least two of the predicted microRNA candidates (cmiRNA9 and cmiRNA2) are expressed in floral or vegetative tissues, and that their corresponding genomic precursor fold back in a typical hairpin structure. cmiRNA9 and cmiRNA2 have both several binding sites in the 3' UTR of a mRNA encoding a UBP1-like protein in Arabidopsis. UBP1 is an oligourydilate specific RRM protein from Nicotiana plumbaginifolia that functions in nuclear pre-mRNA maturation by stimulating splicing efficiency of suboptimal introns and increasing the steady-state level of reporter RNAs. We have identified the potential animal and plant miRNAs homologous to cmiRNA9 and their corresponding RRM mRNA targets in rice, tobacco, C. elegans and Homo sapiens. These results suggest translational repression of RNA binding proteins by microRNAs is conserved in animals and plants.

Genome-Wide Examination of Gene Expression in Developing Maize Anthers

Skibbe, David; Nettleton, Dan; Borsuk, Lisa; and Schnable, Patrick
Iowa State University

T41

A maize spikelet consists of an upper and lower floret, each of which typically contains three anthers. Although anthers in the lower floret are developmentally delayed two to three days relative to those in the upper floret, anthers in both florets proceed through the same developmental stages, and are indistinguishable at the gross anatomical level (Hsu et al., 1988; Hsu and Peterson, 1991). We have established that anthers in the upper and lower florets respond differently to mutations in *rf2a* (Liu et al., 2001). Upper floret anthers of plants homozygous for *rf2a* mutant alleles develop normally, while anther development is arrested in the lower florets of such plants.

To test the hypothesis that anthers in the upper and lower florets express unique sets of genes that cause them to be physiologically and/or developmentally distinct, RNA was isolated from upper and lower floret anthers at six stages of microsporogenesis and analyzed using a spotted microarray that contains 12,000 cDNAs. In total, 48 chips were hybridized.

Analyses of these experiments are still underway, but comparisons between upper and lower floret anthers at the same stage of development have already identified between 68 and 168 statistically significantly (p=0.001) differences in gene expression per stage at five of the six stages of development. Tests were also performed to detect changes in gene expression across the six developmental stages in the upper floret anthers or in the lower floret anthers. Approximately 110 and 300 statistically significant differences were identified in the Tetrad vs. Early Microspore and Early Microspore vs. Mid-Microspore comparisons, respectively Interestingly, only 23% and 57% of the significant differences across development were common between the upper and lower floret anthers at the tetrad and early microspore stages, respectively. These results are consistent with our earlier findings (Liu et al., 2001), in that they suggest that the upper and lower floret anthers are physiologically distinct. To confirm and extend these results, laser microdissection (Nakazono *et al*, 2003) is being performed on the tapetal cell layer of developing anthers. RNA extracted from the cells will be subjected to RT-PCR.

Photosynthetic Mutant Library: Functional Genomics of Chloroplast Biogenesis

Walker, Nigel {1}; Barkan, Alice {1}; Belcher, Susan {1}; Stern, David {2}; Benchley, Jamie {2}; and Harris, Faith {2}
{1} University of Oregon; {2} Boyce Thompson Institute

T42

Chloroplast biogenesis, a process central to plant growth and development, involves a complex interplay between the nuclear and plastid genomes. We have developed a comprehensive genetic resource in maize to facilitate the dissection of this process. Many hundreds of nuclear genes are involved in the assembly, regulation, and function of the chloroplast. However, only a small fraction of these genes have been studied in any detail. Our collection consists of ~2200 transposon-induced mutants with abnormalities in photosynthetic pigmentation, along with data on chloroplast protein, and chloroplast RNA levels in these mutants. Lineage information and phenotypic data for the collection can be found online at http://chloroplast.uoregon.edu.

Mutants contributed to the collection were selected from *Mu*-active maize lines based upon their chlorophyll-deficient leaves or high chlorophyll fluorescence. Previous studies have shown that such mutants may have lesions in many aspects of chloroplast biogenesis, including such processes as protein import and intra-organellar protein sorting, lipid, pigment, prosthetic group

synthesis, chloroplast gene expression, and assembly of the photosynthetic apparatus. The collection is available as a PCR-based reverse-genetic screening service for those wishing to determine the function of genes with known sequence suspected to be involved in chloroplast biogenesis. It is also being used as a forward genetic resource for gene discovery.
Effort is currently directed toward developing rapid methods for linking each mutant phenotype with the corresponding nuclear gene, and for linking predicted nucleic acid binding proteins to their nucleic acid targets in the chloroplast. To such end, we are building a database of RNA binding proteins, using the Rice and Arabidopsis genomes to leverage the available Maize sequence, and targeting those genes that are potentially chloroplast localized.

Extensive cis-Acting Regulatory Variation and Expression Overdominance in Maize: A Molecular Basis for Heterosis

Cattonaro, Federica {1}; Pea, Giorgio {2}; Amadeo, Stefano {1}; Pè, Enrico {2}; and **Morgante, Michelle** {1}

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T43

Variation in coding sequence is classified as synonymous and non-synonymous, reflecting nucleotide variants with and without an effect on protein primary structure and can be recognized directly from DNA sequence. Variation in non-coding cis-regulatory DNA sequences, which affects gene expression levels, has also been proposed as a major component of the genetic basis for phenotypic evolution. In contrast to the former, the latter type of variation can not be recognised from nucleotide sequences alone.

A method to detect changes in transcript levels, due to cis-acting sequence differences, without the necessity to recognize specific regulatory variants (which can be hundreds or even thousands of bases upstream from the transcription unit), has been used in maize for measurement of allelic levels of gene expression. The method involves the study of the relative expression levels of two alleles of a gene in a heterozygous individual through the analysis of SNP markers located in the transcript. We compared the expression of alleles from two maize inbred lines (B73 and H99) in the two reciprocal F1 hybrids.

We analysed initially 12 genes expressed in seedlings and revealed abundant cis-acting variation in maize: 7 out of 12 (58%) genes tested showed greater than 1.5 fold (range: 2.1-4.0) differences in expression among alleles in seedling mRNA, with no significant difference among reciprocal hybrids. The level of this allelic variation we have detected is remarkably higher than that found in the mouse genes where only 4 out of 69 genes (6%) showed allelic differences in gene expression. The differences in expression were confirmed whenever multiple SNPs could be assayed for the same transcript. We then extended our analysis of allelic differences in expression levels to other tissues (leaves, immature ears, kernels, roots) to see if expression ratios remain constant across tissues and/or developmental stages. We observed significant variations in allelic expression ratios across the different RNA samples. In a few cases we observed what we call expression overdominance, i.e. a different allele is most highly expressed in different tissues. Kernels, which are mostly composed of the triploid endosperm, show cases of parental imprinting, with total silencing of the paternal allele.

Cis-regulatory variation appears to be very abundant and widespread in maize. The two inbred lines we analysed are representative of standard maize germplasm and the sample of genes we took is more or less random. The very high levels of cis-acting variation in maize genes are consistent with recent discoveries showing very high levels of sequence diversity in maize transcribed and promoter regions, lack of sharing of repeats in intergenic regions in maize

inbred lines and influence of (retro)transposons insertion/deletions on expression of flanking genes in maize and wheat. Beside representing an important source of phenotypic and quantitative variation, regulatory variation may also provide a possible molecular explanation of the heterosis (hybrid vigor) phenomenon in maize. The patterns of cis-regulatory variation we observed in a sample of maize genes are compatible with both dominance and overdominance, which have been in turn proposed as the major genetic cause of the heterotic phenomenon.

POSTERS

Biochemical Genetics Posters

Correlation and Path Analysis of Grain Yield and its Components in Maize

Abdmishani, Cyrus; Vaezi, S.H.; Yazdi-Samadi, B; and Ghannadha, M.R.
University of Tehran

P1

The efficiency of a breeding program depends mainly on direction and magnitude of association between yield and its components. The purpose of this study was to describe the application of correlation and path analysis to grain yield (GY) in maize. Six generations including two parents and their progenies (F1, F2, BC1 and BC2) were grown in Karaj. Genotypic and phenotypic correlation for GY and its components were calculated. GY was significantly and positively correlated to ear weight (EW), ear circumference (EC), ear diameter(ED), 300-kernel weight (KWT) and number of kernels per row (NKR). Path analysis for GY showed that KWT and kernel depth (KD) had the highest positive effect on GY. However, the ED had negative indirect effect on GY through some traits. It had a positive correlation with GY due to indirect effects through KWT and KD. Path analysis for KWT and NKR showed that ED had high positive direct effect on NKR. Sequential path analysis of six trait components studied revealed that KWR had the highest significant effect on GY but NKR and KW had an indirect effect through KWT on GY. The comparison of path analysis with all correlations and sequential path analysis showed that the two methods lead to nearly the same results and KWT and NKR were the most important yield components.

Characterization of the Plastidic Isoprenoid MEP Pathway in Maize

Arroyo Becerra, Analilia; Ducoing, Helena Porta; Roque, Carolina san Roman; and Leon, Patricia
Instituto de Biotecnologia, UNAM

P2

Isoprenoids constitute one of the largest family of biologically active compounds in all organisms. In plants, isoprenoids participate in a variety of processes including photosynthesis, development, biotic and abiotic responses. All isoprenoids derive from the building units isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMPP). In plants these two main units are synthesized by two pathways, each present in a different cellular compartment: the well known MEV (MEValonate) pathway present in the cytoplasm and the MEP (2C-Methyl-D-Erythritol 4-Phosphate) pathway carried out in plastids. In contrast to the MEV pathway which has been extensively studied, the molecular characterization and the regulation of the MEP pathway in plants is in its infancy.

Maize is one of the most important crops, and they produce a diversity of isoprenoid compounds derived from the MEP pathway. These isoprenoids have biotechnological potential like the antioxidants tocopherols and carotenoids, and also others of basic interest like phytohormones and photosynthetic pigments, that participate in essential processes such as growth, development, stress responses and photosynthesis. Most of the knowledge of this pathway in plants derives from Arabidopsis, so it is our interest to analyze the function and regulation of the MEP pathway in more complex and specialized plants like maize, where it is basically unknown.

In this work we present the isolation and characterization of two maize genes that share high similarity with the Arabidopsis DXS1/CLA1 gene: ZmDXS1 and ZmDXS2. AtCLA1 encodes for the 1-deoxy-D-xilulose 5-phosphate synthase (DXS1) protein which catalyses the first step of

the MEP pathway, and in Arabidopsis seems to be a limiting enzyme for this metabolic route. An expression analysis of both maize genes was carried out through the plant development under several conditions. We observed that ZmDXS1 and ZmDXS2 are expressed in most of the plant tissues, and are positively regulated by light. Remarkably, ZmDXS1 transcript was expressed at higher levels in leaves, whereas ZmDXS2 is enriched in roots, showing a differential regulation in such tissues. In addition, the expression of both genes is regulated by developmental signals in both leaves and roots. Interestingly it was found an important expression level of both genes in the leaf sheaths compared to the leaf blades of the plant, indicating that specific tissue or cellular signals influence the expression of ZmDXS1 and ZmDXS2 genes.
Finally, in an attempt to extend this analysis to the protein level, inmunodetection assays were carried out against total protein extracts from maize plants using heterologous antibodies raised against all the Arabidopsis MEP proteins. The results of this comparative analysis will be presented.

Characterization of the OPT Gene Family in Rice

Bafuma, Pat; and Lubkowitz, Mark
Saint Michael's College

P3

Small peptides of 2-5 amino acids can be used by cells as a nitrogen or amino acid source. Small peptides are imported into cells in an energy dependent manner through the use of a peptide transport proteins located in the plasma membrane. In plants, these transporters are responsible for the distribution of nitrogen, which is often the limiting factor in plant growth. Nitrogen is vital to plant development as it is found in such compounds as chlorophyll, amino acids, nucleic acids, and proteins. Two transport systems have been identified in dicotyledonous plants that translocate peptides of 2-5 amino acids. These systems are the OPT gene family (Oligopeptide Transport) which translocates peptides of 3-5 amino acids and the PTR gene family (Peptide Transport) which translocates peptides of 2-3 amino acids. Whereas members of the PTR family are found in prokaryotes and eukaryotes, OPT genes are only found in plants and fungi. Work recently completed in Arabidopsis thaliana suggests that OPT genes may play a large role in not only nitrogen allocation but nutrient partitioning during developmental processes. Furthermore, several OPTs have been shown to transport the tripeptide glutathione, an important source of organic sulfur. Despite the agricultural importance of monocots and the importance of resource acquisition and partitioning in plants, no study has been conducted of OPT genes in a monocot. To this end we have identified nine putative rice OPTs which we intend to initially characterize through heterologous expression in yeast. To date, we have amplified one of these genes, OsOPT1, out of a cDNA pool, successfully cloned it into a yeast expression vector, and transformed our construct into an OPT yeast mutant. We are currently investigating various tetra- and pentapeptides substrates of OsOPT1.

Molecular Mapping of QTL for Fall Armyworm Resistance and Associated Traits in a Tropical RIL Population (CML67xCML131)

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P4

Maize production in tropical ecologies can be severely reduced by fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith). Host plant resistance is the cornerstone of integrated pest management, with tropical maize varieties being developed at the International Maize and Wheat Improvement Center (CIMMYT) with resistance to stem borers and armyworm. QTL for

resistance to stem borer and earworm have been identified in recent years but little information is available on the genomic regions associated with FAW resistance and the phytochemical bases for this resistance. We analyzed quantitative trait loci (QTL) involved in resistance to FAW in a recombinant inbred line (RIL) population from the cross CML131 (susceptible) X CML67 (resistant). RFLP linkage map developed previously (Groh et al., 1998) was used for the QTL analysis and detected by the method of composite interval mapping (CIM). Resistance was evaluated as leaf feeding damage (LFD) under artificial infestation in three environments. Leaf toughness (LT), leaf thickness (LTH), and chlorophyll content (CC) were evaluated as putative components of resistance. Seven major QTL in bin 1.06/11, 5.06, 7.03, 8.02/6, and 9.03 explained 49% of the phenotypic variance (σ^2_p) for LFD. All alleles contributing to reduced LFD originated from the resistant parent. Three QTL located in bin 1.06/11 and 8.06 were determinated to affect LT and explained 27% of $\sigma^2_{p.}$ One QTL was located for LTH in bin 8.04 and two QTL (46% of σ^2_p) were identified for CC in bin 3.05 and 4.08. QTL identified for FAW resistance clustered with LT in bin 1.06, 1.11, and 8.06, while LPC clustered with FAW resistance in bin 1.06. Chlorophyll QTL did not overlap with insect resistance factors. QTL for cell wall bound phenolic acids were determined previously (Ramputh, 2000) and compared with QTL for FAW resistance. Resistance in bin 1.11 clustered with QTL for *trans*-ferulic acid, 8-5' diferulic, and 8-5' diferulic acid. FAW resistance was best explained by three QTL for leaf toughness, with QTL in bin 1.11 also clustering with QTL associated with cell wall phenolics. This observation is consistent with a broad-based, structural resistance mechanism previously reported for tropical maize resistant to lepidoptera field pests.

Groh et al., 1998. Crop Sci. 38:1062-1072.
Ramputh, A. 2000. Ph.D. Thesis. University of Ottawa, Ottawa. ON.

How High Altitude Maize Landraces Respond to Ultraviolet Radiation - Investigation of Different Mechanisms Involved in UV-B Acclimation

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P5

UV-B radiation is a natural component of sunlight, constituting about 4% of the total solar energy reaching the earth's surface. There is global concern about increased UV-B levels because depletion of stratospheric ozone significantly increases penetration of this damaging radiation. Land plants are particularly sensitive, because photosynthetic tissues are inevitably exposed to UV-B, resulting in damage that must be repaired to maintain cellular functions. The fluence rate of this natural component of sunlight varies significantly with altitude and with proximity to the equator and to the poles. Consequently, analysis of maize normally grown above 2000 m, should provide insight into acclimations and adaptations from natural selection at high UV-B fluence; these lines may yield alleles useful for maize breeding and define the processes that confer UV-B tolerance. In the current project we studied UV-B responses in five high altitude landraces of maize: Andean landraces Confite Puneco and Mishca and Mexican lines Conico, Arrocillo Amarillo, and Cacahuacintle. First, we tested cellular functions that are known to be affected by UV-B to compare the responses in these lines with a b, pl W23 line lacking anthocyanins. We previously found this line to be very sensitive to UV-B. Our results show that different amounts of UV-B induced DNA damage accumulate in the lines, but independently of the altitude were they usually grow. In terms of sunscreens, however, the high altitude landraces have high levels of two UV-B inducible flavonoids in leaves, which were identified as maysin and rhamnosylioorientin. These flavonoids are detected at very low levels in near-isogenic W23 lines, varying in B and Pl transcription factor expression. To gain a more

global view of UV-B responses we conducted transcriptome analysis to compare UV-B responses in the high altitude lines to the responses in W23 lines. Microarray hybridization experiments were done with landrace samples that were field-grown in summer 2002 next to b pl W23 at Stanford. We used three regimes: sunlight, no UV-B, and supplemental UV-B equal to 6X current fluence rate. Short-term UV-B exposure under greenhouse conditions was also assessed. From the transcriptome profiling and other attributes measured, it appears that maize plants may have independently evolved various pathways that can confer better tolerance to increased levels of UV-B present in their natural environments. In a separate set of experiments we documented that shielded tissues such as immature ears or leaves wrapped in UV-B opaque plastic exhibit transcriptome changes after leaf irradiation. These data suggest that a signal(s) from irradiated tissues generates a systemic response to UV-B. Supported by grants from the National Science Foundation and USDA.

Proteomic Profiles and Nutritional Properties of Maize Landraces of 'El Bajio'

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P6

In Mexico, maize is the most extensively cultivated crop and is the major grain produced in the country (Food and Agriculture Organization, 2002 FAO STAT database results).

'El Bajio' is one of the main agricultural region and includes the low lands (1500-2000 m above the sea level) from the states of Queretaro, Guananjuato, Michoacan and Jalisco. In terms of cultivated area, 'El Bajio' is one of the largest maize growing regions. In more than 85% of the land (around 800 000 ha), maize landraces are cultivated, under rain fed conditions. Whereas in the remaining 15%, commercial hybrids are grown, under more favorable conditions of soil, rain and/or irrigation.

A research group at INIFAP-Celaya, are collecting maize landraces from distinct regions of 'El Bajio'. After collecting hundreds of plants, they have found so far 10 most frequent races, based on phenotypic criteria. Using this information, our goal is to create a data base with environmental, physiological, biochemical and molecular data, in order to valuate the genetic variability of these maize landraces.

We are currently analyzing nutritional and proteomic aspects of this germplasm collection. These studies include: a) the total protein content, b) the zeins levels, c) the lysine concentration and d) proteomic profiles through 1D and 2D PAGE. The proteomic profiles will be compared through digital analysis in order to obtain a reproducible profile of each landrace.

Substrate Specificity of the Rice Peptide Transporter OsPTR1

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P7

Plant growth and reproductive success depends upon acquiring and distributing nitrogenous compounds. This allocation of nitrogenous compounds involves many transporters including amino acid transporters, nitrate transporters, ammonium transporters, purine transporters, and peptide transporters. Of these transport systems, peptide transporters remain the least characterized. Peptide transport is the ability of cells to transport peptides across membranes in an energy-dependent manner. Members of the PTR gene family (Peptide Transport) translocate peptides of 2-3 amino acids, belong to the Major Facilitator Superfamily, use the proton motive force to energize translocation, and have been identified in prokaryotes and

eukaryotes. AtPTR2b, an Arabidopsis transporter, is expressed in root epidermal cells, cortical cells, and vascular tissue, and AtPTR2b mutants arrest during seed development. These observations suggest that AtPTR2b plays a critical role in nitrogen partitioning through the translocation of small peptides. We have identified an AtPTR2b ortholog, OsPTR1, in the rice database. We are in the process of determining if this gene encodes a peptide transporter. We used PCR to amplify OsPTR1 from a rice seedling cDNA pool and cloned the resulting product into a yeast expression vector. This construct, pRPT1, has been transformed into a yeast peptide transport deficient strain and we are in the process of determining if OsPTR1 can complement the ScPTR2 mutation.

Comparative Study of Lepidopteron Resistance in Maize Lines through Protein Analysis

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P8

Both the fall armyworm (FAW) and the southwestern corn borer (SWCB) have been known to cause significant yield loss in maize through the destruction of the whorl stage leaf. To date, several quantitative trait loci (QTL) corresponding to resistance from damage during the whorl-stage have been identified. Previously, protein patterns from parental lines that are resistant (Mp705) or susceptible (Oh28) to fall armyworm were analyzed through 2-dimensional gel electrophoresis. Comparisons were made focusing on the presence/absence and relative intensity of protein spots. Differentially expressed proteins were identified through conventional methods (Soto, unpublished). For a more comprehensive study of these proteins the midgut of the FAW and SWCB exposed to both the resistant (Mp705) and susceptible (Oh28) lines through artificial diet was extracted and analyzed for protein content. In addition preference tests were run to see if the Lepidopteron were able to sense the susceptibility of the tissue before feeding on it. The resulting data showed that *Glossy15* and *Glossy8* are genes that are directly correlated with Lepidopteron resistance. A wax reversal preference test was run to identify the location in which the gene is expressed (i.e.: wax or leaf tissue). The goal of this comparative protein research is to identify proteins associated with Lepidopteron resistance in maize lines by narrowing the number of proteins found in the leaf extraction studies. These resistance proteins can then be integrated into maize lines in order to reduce the damage caused by these insects as well as reduce the cost of pesticides.

Evolution of an Amine Oxidase for Detoxification of Fumonisins by Gene Shuffling

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P9

Fumonisins are mycotoxins produced by the maize pathogen Fusarium verticillioides. The widespread occurrence and toxicity of fumonisins to certain livestock has focused interest on methodologies for reducing fumonisin levels in maize grain. The isolation of genes from fungi capable of metabolizing fumonisins presents the opportunity for application of a transgenic approach to degradation of fumonisins in infected tissue. An amine oxidase identified as a potential candidate for catalyzing a single-step fumonisin detoxification reaction exhibits low specificity towards fumonisins, and little activity in the milieu of maize apoplast. Here, we describe the evolution of the amine oxidase by gene shuffling for reactivity towards fumonisin B1 in the maize apoplast. Four iterations of gene shuffling generated a variant amine oxidase

with high specificity towards fumonisin B1 and its isomers, a dramatic increase in kcat/KM at the apoplastic pH of 5.5, and the ability to detoxify exogenously applied fumonisin B1 when targeted extracellularly in planta.

Functional Characterization of Evolutionary Conserved MYB Domain Residues Using P1 as a Model

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P10

MYB domain proteins are conserved transcriptional regulators that are found in all eukaryotes. These transcriptional regulators include the widely distributed R1R2R3 MYB proteins represented in animals by the c-MYB proto-oncogene and in plants by both the pc-MYB and the R2R3 MYB gene families, the latter of which has expanded dramatically in higher plants. Some residues within the MYB domain have remained absolutely conserved in all eukaryotes while others are conserved only in the higher plants R2R3 MYB proteins. We have utilized the Zea mays P1 protein, which belongs to the recently expanded P-to-A clade, to investigate the functional and structural roles of several residues found within the MYB domain. These residues are either highly conserved among all MYB domains or uniquely present among some MYB sub-families. Our studies focused on the Cys53, which is absolutely conserved in all MYB domains, and Cys49, which is present in just the typical R2R3 MYB domains, respectively. In addition, we also investigated the significance of the Pro63 change to Ala, a landmark of the P-to-A group of R2R3 MYB proteins in the grasses. Other residues characteristic to all plant R2R3 MYB proteins include the replacement of the first Trp of the R3 MYB repeat by a hydrophobic amino acid and the insertion of a Leu residue in the R2 MYB repeat. The significance of these two residues was also investigated. Our studies provide surprising insights on how function has been influenced by the single amino acid changes that shaped the evolution of the MYB gene family.

Characterization of a Maize Inhibitor of Aflatoxin Accumulation

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P11

Pre-harvest infection of maize ears by the filamentous fungus Aspergillus flavus is a recurrent problem that reduces marketable grain yields. In addition, infection threatens human health because of the production of aflatoxin, a highly carcinogenic mycotoxin. The development of agronomically desirable maize cultivars with durable resistance to A. flavus infection and aflatoxin contamination is a promising approach for reducing this problem. Huang et al. (Phytopathology 87: 622-627) identified independent activities that were inhibitory to fungal growth and aflatoxin production in kernels of Tex6, an inbred with resistance to A. flavus (Hamblin and White, Phytopathology 90: 292-296). We are using a liquid culture bioassay to purify antifungal maize kernel proteins as a first step toward obtaining markers that can be used to follow the segregation of resistance in marker-assisted breeding programs. Selective (NH4)2SO4 precipitation, gel filtration chromatography and DEAE anion exchange chromatography have allowed us to separate the growth inhibition activity from the inhibition of aflatoxin biosynthesis. Additional purification steps and characterization of the aflatoxin biosynthesis inhibitor protein (ABI) are in progress.

Characterization of an OPT Type Transporter from Zea mays

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P12

Peptide transport is defined as the translocation of peptides 2-5 residues in length across the plasma membrane in an energy dependent manner. In lower eukaryotes internalized peptides serve primarily as a source of amino acids for protein synthesis or as a nitrogen source. In plants two gene families have been identified that mediate the import of small peptides: the Peptide TRansport family (PTR family) and the OligoPeptide Transporter family (OPT family). The PTR family translocates peptides 2-3 amino acids in length and is present in prokaryotes and eukaryotes. OPT genes, on the other hand, are only found in plants and fungi and import peptides of 3-5 residues. The most characterized plant OPT gene is AtOPT3 from Arabidopsis. This gene is expressed in vascular tissue and loss of function mutants exhibit an embryo lethal phenotype. We have identified several putative OPT type transporter in Zea mays using orthologous sequences from yeast and Arabidopsis. We are in the process of characterizing one of these genes named Zmopt3. We are using heterologous expression in yeast to determine if the zmopt3 gene product is able to translocate various peptide substrates. Furthermore, we have determined that zmopt3 is expressed at relatively high levels in developing leaves, embryos, and meristems.

Gene Duplication in the Carotenoid Biosynthetic Pathway Preceded Evolution of the Grasses (Poaceae): Implications for Pathway Engineering

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P13

In plants, the biosynthesis of carotenoids occurs on membranes of chloroplasts, chromoplasts, and amyloplasts, genetically identical plastids of very different internal membrane architecture, a critical characteristic not generally considered in breeding or metabolic engineering efforts. The plant enzymes, which are for the most part well established, are encoded in the nucleus and targeted to the plastids. Despite ongoing research on carotenoid biosynthesis in model organisms or carotenoid accumulating flowers, there is a paucity of information on pathway regulation operating in plants of world-wide agronomic importance, most specifically in the grasses (Poaceae). As a result, efforts to either breed for or metabolically engineer improvements in carotenoid content or composition in cereal crops have led to unexpected results because of the insufficient understanding of how metabolon assembly is controlled in plastids of different membrane architectures. The biosynthesis of all carotenoids begins with the formation of the 40-carbon backbone, phytoene, a step mediated by phytoene synthase (PSY). In maize endosperm, carotenoid content positively correlates with the dosage of the PSY structural gene, Y1. In comparison to maize, rice accumulates no endosperm carotenoids, despite having an obviously functional PSY in green tissue. To better understand why these two related grasses differ in endosperm carotenoid content, we began to characterize the genes encoding PSY, since this enzyme appeared to catalyze a rate-controlling step in the pathway. Previous cloning of the maize Y1 locus, established this gene to encode PSY on the basis of sequence homology with other known phytoene synthase genes, though function of the gene product had never been demonstrated. Most plants, tomato being an exception, have single genes encoding PSY and this was long thought to be true for maize and as a corollary, rice. We present evidence that both maize and rice possess duplicate PSY genes encoding functional but unique enzymes. Furthermore, the PSY duplication is prevalent throughout the grasses

(Poaceae), suggesting that this genetic event preceded the evolution of the Poaceae. These findings will have broad impact both on investigations of grass phylogeny and on breeding of enhanced carotenoid content in an entire taxonomic group of plant crops critical for global food security.

Anthranilate Synthase from Agrobacterium tumefaciens *Promotes Increases in Free Tryptophan When Expressed in Plant Seeds*

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P14

Soybeans and corn represent important sources of protein for both human and animal nutrition. However, because these two crops are deficient in certain essential amino acids, livestock feed formulated from soy and corn is not nutritionally balanced unless higher cost additives are included. A lower cost alternative involves engineering crops to accumulate high levels of the desired amino acids. One strategy to increase tryptophan content in seeds is to make the tryptophan biosynthetic pathway less sensitive to end-product inhibition. Anthranilate synthase (AS) catalyzes the first committed step in tryptophan biosynthesis, and this enzyme is feedback-inhibited by tryptophan. In plants, AS is a two subunit enzyme that is localized in the plastid. The alpha subunit catalyzes the conversion of chorismate to anthranilate with ammonia as a co-substrate. The beta subunit is an amidotransferase that provides ammonia, from glutamine, to the alpha subunit. In certain bacteria, however, the chorismate conversion and glutamine amidotransferase activities of AS are contained on a single polypeptide. We have previously identified the gene for such an enzyme from Agrobacterium tumefaciens, and described the design and construction of variants of A. tumefaciens AS that are less sensitive to feedback inhibition (Weaver et al., Patent Application Publication US20030097677A1). Here we show that seeds from transgenic plants expressing these feedback-insensitive forms of A. tumefaciens AS (also engineered to contain a plastid targeting signal) accumulate high levels of free tryptophan, and that higher levels are observed in comparison to transgenic seeds expressing a feedback-insensitive allele of the maize AS alpha subunit.

Amarantin Accumulation in Transgenic Tropical Maize Germoplasm

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P15

An amaranth (Amaranthus hypochondriacus) 11S globulin cDNA, encoding one of the most important storage proteins (amarantin) of the seed, with a high content of essential amino acids, was used in the transformation of CIMMYT tropical maize genotype. Constructs contained the amarantin cDNA under the control of a tissue specific promoter from rice glutelin-1 (osGT1) or a constitutive (CaMV 35S) promoter with and without the first maize alcohol dehydrogenase intron (AdH). Southern blot analysis confirmed the integration of the amarantin cDNA, and copy number ranged from one to more than 10 copies per maize genome. Western blot and ultracentrifugation analyses of transgenic maize indicate that the expressed recombinant amarantin precursors were processed into the mature form, and accumulated stably in maize endosperm. Total protein and some essential amino acids of the best expressing maize augmented 32% and 8-44% respectively, compared to non-transformed samples. The soluble

expressed proteins were susceptible to digestion by simulated gastric and intestinal fluids and it is suggested that they to show no allergenic activity. These findings demonstrate the feasibility of using genetic engineering to improve the amino acid composition of grain crops.

Evolution of Novel Gene Function by Divergent Targeting of Duplicated Gene Products
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P16
The tetraploid ancestry of maize and related species has resulted in a genome consisting of many collinear chromosome segments carrying duplicated genes. The retention of such a high proportion of these duplicated genes is likely to derive from a variety of selection mechanisms. Our studies of the duplicate maize genes cpx1 and cpx2 indicate that they have diverged in function by a change in protein targeting that is unique to the Maydeae. CPX encodes an enzyme for chlorophyll and heme biosynthesis, in a pathway that has been found exclusively in the plastids of higher plants. A duplicated gene in maize and its close relative encodes the same enzyme, but with a novel N-terminus and an absence of plastid targeting. Gene fusion experiments indicate that the CPX2 product localizes to mitochondria in maize. Other Zea species also have both forms of the gene, while different classes of monocots do not. Mutant analysis in maize using TUSC shows different functions for the two CPX enzymes.

An Introgression Library of the Maize Early-Flowering Variety Gasp Flint into B73
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P17
Introgression libraries (ILs) are ideal tools for dissecting the inheritance of quantitative traits as they capture and display the effect of natural allelic variation in a homogenous genetic background, where epistatic effects are reduced to a minimum (Zamir, Nature Rev Genet 2:983 -989). The aim of this research is to produce a maize IL with the genome of the extremely early-flowering variety Gasp Flint introgressed into the elite genetic background of the medium-late line B73. Based on trait differences between the donor and the recipient genotypes, the IL will serve as a permanent source of nearly isogenic material for QTL analysis for many morpho-physiological traits, such as flowering time, plant height, number of leaves, tillering, root architecture, yield and yield components, heterosis, etc. Specific chromosome segments could also be utilized in marker-assisted selection programmes. The IL is being produced by a molecular marker-assisted backcross based on publicly available SSR markers. The target average Gasp Flint introgressed segment length is ca. 25 cM; this will allow for the production of ca. 70-80 IL lines covering the maize genome. A genetic map based on the starting BC1 population has been generated and used as basis for the selection of flanking markers at defined chromosome segments. Currently, we are producing seeds of the BC5 generation (ca. 98% of B73 genome recovered) which will be grown and selfed during the year 2004. Preliminary QTL analysis showed that a major QTL for flowering time and number of nodes maps on bin 8.05, where QTLs for the same traits have already been mapped (Salvi et al. PMB 48:601-613).

Transgenic Maize Grain Containing Porcine Alpha Lactalbumin Has Elevated Levels of Lysine

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P18

Because maize is used primarily for food and feed, its nutritional value is an important characteristic. One of the main limitations to the nutritional value of maize is its amino acid balance. Certain essential amino acids such as lysine are limiting in maize based diets, while others are in excess. This leads to poor utilization of maize protein and high levels of nitrogen waste. Our objective was to determine if the amino acid balance of maize endosperm could be altered by the expression of the milk protein porcine alpha lactalbumin in the endosperm of transgenic maize. A construct containing a 27 kDa gamma zein promoter and a synthetic coding sequence was used to transform maize by microprojectile bombardment at the ISU plant transformation center. Lines in which the functional transgene segregates as a single dominant locus were characterized. By comparing endosperms from sibling kernels on segregating ears, we determined the effect of the transgene on the amino acid balance of the endosperm in two different transformation events for two generations. The total protein content was significantly higher in transgenic endosperms of one event in one generation, while the lysine content was significantly higher in all cases. These increases in lysine content ranged from 29 to 47%. Other amino acids exhibited consistent though not always significant changes as well, generally reflecting the amino acid composition of porcine alpha lactalbumin. Thus Aspartic Acid and Threonine were increased in transgenic kernels while Proline was reduced. This work illustrates the feasibility of manipulating amino acid balance by production of foreign proteins in the grain.

Phenotypic Analyses of Phytochrome B Single and Double Mutants in Maize

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P19

Plants use the light environment to initiate and sustain developmental patterns. Phytochromes are the principle red and far-red photoreceptors and mediate a number of developmental processes throughout the life of the plant. Seed germination, shoot elongation and time to flowering are all under phytochrome control. The phytochrome gene family has been examined in detail in the model eudicot, Arabidopsis thaliana, and consists of five genes PHYA-E. The gene family structure in monocots consists of only three members PhyA-C. We have recently shown that the maize genome contains a complete duplication of the phytochrome family that map to homeologous regions of the genome. The maize Phy homeologs share 94-98% amino acid identity and are expressed in seedling tissues. Thus, all six Phy genes of maize are likely to encode functional proteins (Sheehan, et al 2004). Using reverse genetics, we have begun the dissection of the PhyB light-signaling pathway. Mu alleles of both PhyB1 and PhyB2 have been identified and are currently being introgressed into multiple genetic backgrounds. Preliminary data suggest that PhyB1 and PhyB2 are not completely redundant in their functions. PhyB1 is solely responsible for inhibiting mesocotyl elongation under red and white light conditions, whereas both PhyB1 and PhyB2 appear to promote flowering. This work suggests that in maize, the PhyB1 and PhyB2 signaling pathways are overlapping but not completely redundant.

Cloning and Characterization of viviparous15: Application of MuTAIL-PCR, Blast Filtering, and In Silico Subtraction to Identify Candidate Genes

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P20

Maize viviparous mutants have been informative to study various developmental and metabolic processes in plants. Most of the known viviparous mutations are ABA deficient due to defects in carotenoid or ABA biosynthesis. The viviparous15 (vp15) mutation causes precocious germination and early seedling lethality. This phenotype is nearly indistinguishable from vp13 mutants (Porch et al., Maize meeting 2003; Tseung et al., this meeting). Genetic complementation tests showed that vp15 is not allelic to vp13. These data suggest that vp15 mutants may have a defect in the same pathway as vp13. The Vp13 locus encodes an enzyme required for Molybdenum cofactor (Moco) biosynthesis. Moco is a cofactor for four classes of enzymatic activities including absicic aldehyde oxidase, the final step in ABA biosynthesis. We used MuTAIL-PCR to clone the flanking sequences of Mutator transposons from the vp15-1 mutant allele. A library of 384 MuTAIL clones was single-pass sequenced. The resulting MuTAIL sequences were then analyzed by three steps in silico: 1) search for unique MuTAIL clones in the vp15 library among MuTAIL libraries from other mutations, 2) search for cereal ESTs and maize gss sequences that have similarity to the MuTAIL sequences by BlastN analysis, and 3) search for annotated proteins by BlastX analysis with the DNA sequences assembled in the second filtering step. By this analysis, one of the unique contiguous MuTAIL clones in the vp15 library showed a significant homology to human MOCS2, the molybdopterin synthase small subunit.. We further confirmed that this Moco biosynthetic gene is Vp15 by mapping two additional allelic mutations to the locus. The vp15-1 and vp15-DR1129 harbor Mu insertions in the coding region of Vp15 whereas the vp15-MJ7546 has a 15 bp deletion in the C-terminal portion. Biochemical analysis shows that the activities of three Moco enzymes (aldehyde oxidases, xanthin dehydrogenase, and sulfite oxidase) are reduced.

Biochemical and Molecular Characterization of Maize vp13 Mutants

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P21

Abscisic Acid (ABA) is necessary for seed maturation and dormancy, and ABA deficiency in maize often results in viviparous seed. Three viviparous loci have been cloned and two are required for ABA biosynthesis (vp14 and vp5), while one is crucial in ABA signaling (vp1). The viviparous13 (vp13) mutant presents a novel phenotype characterized by normal accumulation of carotenoids and a distinctive lethal phenotype at the seedling stage. Five vp13 alleles have been isolated from a number of Mutator populations and introgressed into W22. We cloned the vp13 locus using MuTAIL-PCR to amplify transposon flanking sequences from both mutant and normal siblings. A vp13 specific transposon flanking sequence was used to identify a BAC containing the Vp13 locus. The sequence of this locus shows homology to Cnx1 genes that are involved in molybdenum cofactor (Moco) biosynthesis. We have identified molecular lesions in three of the five vp13 alleles indicating that the maize Cnx1 homologue is the Vp13 gene. Moco is required for the final aldehyde oxidase step of ABA biosynthesis in addition to other enzymes. Consistent with the predicted function of Cnx1 in maize, we show that vp13 mutants lack aldehyde oxidase, xanthine dehydrogenase, and sulfite oxidase activities. In addition

endogenous ABA levels of vp13 mutant seedlings are reduced, and vp13 seedling growth is sensitive to exogenous ABA. These data suggest that vp13 is a pleiotropic biosynthetic mutant and suggest that there is an essential function of one or more Moco requiring enzymes in plants.

Bioinformatics Posters

Spatial Analysis of cDNA Microarray Experiments

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P22

Microarray experiments allow RNA level measurements for many genes in multiple samples. However, mining the biological information from the large sets of data generated by microarrays requires the use of appropriate statistical methods to adjust the observed values for experimentally introduced variability (normalization process) before testing differences among samples. This paper explores the use of spatial analysis to normalize cDNA microarray data and reports on the efficiency of this method for removing experimental biases, a critical step for reducing false positives and false negatives. Application to a set of maize cDNA arrays serves as a case study to validate the spatial adjustment. Results show that the spatial analysis improved the selection of candidate genes.

GDPC: The Genomic Diversity and Phenotype Connection: Accessing Data Sources via XML Web Services

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P23

Research projects on genomic diversity and phenotypes have generated valuable data collections that tend to be abandoned after results are published. Ideally, this data would be made widely available by migrating the data to larger, public databases. The purpose of the Genomic Diversity and Phenotype Connection (GDPC) is to simplify access to data and thereby increase its effective reuse. GDPC is software written in JAVA that works with taxa, loci, environment experiments, genotype experiments, localities, genotypes, and phenotypes. GDPC provides: 1) data sources that are XML web services; 2) programmatic access to data sources; and 3) a front-end application that allows users to retrieve data.

GDPC provides the infrastructure to create connections to data sources that mask the complexities of the data's underlying format/schema. These 'GDPC connections' typically support databases and are designed as web services that transfer XML via the SOAP protocol. 'GDPC connections' can also be designed to access databases using the JAVA JDBC API. Future plans include developing a connection capable of accessing local flat files. Researchers can create connections to their data that allow them to integrate it with public data. Once in the GDPC format, it is possible to integrate, analyze, and view data from multiple sources. This is a significant advantage over tools that access only one source. A 'GDPC connection' already exists to the maize diversity database, Panzea (http://www.panzea.org). Other 'GDPC connections' are being developed, including one to the comparative cereal database, Gramene (http://www.gramene.org). JAVA classes compatible with GDPC are provided to help other organizations make their data 'GDPC enabled.' Future plans include registering 'GDPC

connections' with MOBY Central (http://www.biomoby.org) to allow users to locate them via that directory service.
Several research projects make their data available via web sites, but programmatic ways to retrieve data generally do not exist. In contrast, GDPC provides a JAVA API that standardizes access to data sources. Programmers can use this API to develop 'GDPC aware' front-end applications that perform algorithms relevant to their project. The GDPC Browser and TASSEL (http://www.maizegenetics.net/bioinformatics/tasselindex.htm) are applications that currently use this API. The planned development of a Mesquite module (http://mesquiteproject.org) will make any 'GDPC connection' accessible from the Mesquite toolset.
The GDPC Browser is a front-end application that allows users to retrieve, view, and group data based on property values. With this application, users can connect to a data source(s) and retrieve data based on user defined search criteria. The data properties can be viewed by selecting the individual data elements. Working lists of these elements can be created and sorted based on user needs. These working lists can be saved/opened as XML files. Data can be exported to other formats (i.e. PHYLIP) chosen by the user. This tool and other analysis tools will facilitate QTL linkage mapping and association mapping on a grass and genome wide level.
The source, binaries, documentation, etc. are freely available at:
www.maizegenetics.net/gdpc/index.html.

MaizeGDB Curation and Undergraduate Training: Can They Be Symbiotic?

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P24

MaizeGDB has recently developed and released web-based curation tools giving community members the ability to curate database records thereby permitting researchers to enter their own data directly. Using undergraduates as community curators benefits MaizeGDB by providing a competent 'labor force' to enter data from published literature and the students benefits include but are not limited to: 1) gaining experience in using database tools; 2) having the opportunity to learn to interpret primary literature; and 3) gaining insight into the field of maize genetic and genomic research. As a pilot project, a group of undergraduate biology majors at Kentucky Wesleyan College has been trained to use the curation tools. Students identify data germane to maize genetics and genomics from primary literature, and enter those citations along with appropriate information into the database. The supervising professor evaluates each student's ability to glean relevant data from the literature and to enter citations and data into the database both accurately and efficiently. Professional curators at MaizeGDB evaluate the accuracy of records entered by students before releasing them to the public.

PGROP: the Plant Genome Research Outreach Portal

Baran, Sanford; Lawrence, Carolyn; and Brendel, Volker
Iowa State University

P25

As more plant genome research outreach resources become available, efficient methods for finding the right resources to suit a particular individual's needs must be created. The Plant Genome Research Outreach Portal (PGROP) site serves as a gateway to online educational activities, programs, and resources about plant genomics. PGROP provides access to information that addresses the needs of a wide-ranging audience including high school, undergraduate, and graduate students; teachers; university faculty; and the general public. Also available through the site are resources for locating grants and fellowships, internships, and

various other opportunities in the field of plant genomics research. PGROP can be accessed online at http://www.plantgdb.org/pgrop. You will discover a wealth of information and tools at your fingertips when you walk through the portal!

Identifying microRNAs in Plant Genomes

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Cold Spring Harbor Laboratory

P26

The ability to control gene expression during development in plant genomes could be used for improving crop yields, resistance to disease, and environmental adaptability. It has been suggested that microRNAs, or miRNAs, control developmental processes such as meristem cell identity, organ polarity, and developmental timing by interfering with the expression of mRNAs. MicroRNAs are 20 to 22 nucleotide sequences that arise from larger precursor sequences transcribed from non-protein-coding genes. The precursors form a hairpin structure which is processed by a ribonuclease III-like nuclease known as DICER-LIKE (dcl1) to form the mature products. Unfortunately current algorithms for detecting these precursors, and their potential miRNA, are not as robust as protein-coding gene prediction tools. Patscan is a pattern matching program for nucleotide sequences that allows for the RNA basepairing and can be used to detect potential stem-loop forming sequences. Due to the large quantity of hairpins in the initial candidate set found from Patscan, filters were applied to eliminate sequences that match the hairpin pattern yet fail to meet properties found amongst known miRNAs. Initial analysis was conducted on the Arabidopsis genome. Since many of these noncoding RNA display sequence conservation amongst eukaryotic genomes, we expect that some of the miRNAs found in Arabidopsis will be conserved in other plant genomes, such as maize and rice. Using Blast we compare our initial candidate set in Arabidopsis to other plant genomes, removing all hits that lack a hairpin structure and sequence conservation between the stems. Experimental verification of these remaining hairpin sequences will demonstrate which are actual miRNAs. The hairpin stems can then be used to identify the corresponding target gene for each miRNA. I will present preliminary results of this work in the poster.

MAZORKA: A Fully Automatic Bioinformatics Process for Maize ESTs

Martinez, Octavio
CINVESTAV

P27

An automatic bioinformatics process was developed for the capture and curate of EST sequences from complete and subtractive maize libraries. The system was implemented using shell and Perl scripts and a MySQL data base into a cluster computer under Linux. Also a web based user interface was implemented that permits simple as well as complex queries to be performed. The design and implementation of the system is presented and discussed underling the difficulties and pitfalls encountered.

Informatics Filtering and Cluster Analysis of MuTAIL Sequences: Tools for In Silico Detection and Confirmation of Transposon Tagged Mutants

McCarty, Donald R.; Wu, Shan; and Latshaw, Sue
University of Florida

P28

Robertson's Mutator has proven to be a powerful tool for insertional mutagenesis in maize. The desired mutation frequencies obtainable in highly actively lines are typically associated with

presence of a high copy number of Mu elements. The copy number problem strongly limits scalability of conventional approaches for molecular analysis of transposon tagged mutants. Hence, we have sought to develop genomics scale strategies that leverage the full power of high copy insertions such as Mu. We have used high throughput sequencing of cloned MuTAIL PCR products to analyze a diverse set of Mu tagged seed mutants isolated from the UniformMu population. An informatics pipeline was developed to facilitate mapping and annotation of sequence tagged Mu insertions with respect to all available the maize genome resources. Repeat masking excluded repetitive sequences and contaminating organelle DNA from the MuTAIl sequences prior to blast analysis. A primary blastn search identifies hits in databases containing cereal EST's, maize gss accessions (excluding MuTAIL sequences) and current maize genome assemblies, respectively. Using current databases, 70% of the MuTAIL gss accessions hit entries in one or more of the target databases below an expectation value cut off of 1 e-25. The resulting set of EST, gss and genome assembly sequences are filtered by repeat masking and annotated by a blastx search of the NCBI nr protein database. The blastn and blastx results are parsed in to a relational database which can be queried at uniformmu.org. A second stage in the analysis parses the MuTAIL sequences in to clusters based on the blastn results. Each cluster resolves the set of libraries that contain the same or related Mu insertion sites (e.g. presumptive alleles). Hence, blast clusters provide a potent filter for identification of candidates for the mutant gene. The efficiency of this filter increases as more mutants are added to the database. Finally, we have constructed a pedigree index for the UniformMu population that facilitates integration of pedigree information for each mutant with the MuTAIL sequence analysis. Applications of these tools to identification of tagged seed mutants will be demonstrated.

Informatics Infrastructure for Performing Field Genetics on a Genomics Scale

McCarty, Donald R.
University of Florida

P29

A centralized relational database for maize genetics and a supporting suite of network enabled graphical interface tools for data entry were developed in support of our Endosperm Functional Genomics Project. The mysql database provides integrated storage, validation and retrieval of field, pedigree, seed inventory and image records for a large field and laboratory based genetics program. Specific task oriented data entry and retrieval tools are fully barcode enabled allowing routine data entry and database navigation operations to be performed with little or no mouse or keyboard input by the user. Supported tasks include primary mutant screen, image documentation, heritability screen, seed inventory and pedigree lookup. In addition, the suite includes a graphical utility for formatting and printing barcode labels using a variety of standard as well as custom templates suitable for labeling DNA sample tubes, microtiter plates, seed envelopes, plant tags and pollination bags, etc. The print utility supports data imported from database tables, spreadsheet files or manual entry. An indexing tool automates construction of a pedigree index from the database. The index assigns a unique segmented number to each culture in the population that encapsulates its lineage allowing, for example, the last common ancestor of any two individuals in the population to be determined at a glance. The resulting pedigree may be represented as a tree that can be viewed and browsed graphically using a java program. The suite of database tools is written in the java programming language and source code is available allowing customization and adaptation whole or in part to other projects.

MaizeGDB: Four Usage Cases

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P30

MaizeGDB is a central repository for maize information for the maize community, combining hand-curated information on maize genes with high-throughput data from laboratories to provide an overall view of maize genetics and genomics with an easy to use interface. We present four distinct cases of using MaizeGDB to answer common questions that maize researchers may have. These cases demonstrate the ease of use and availability of detailed information at your fingertips with MaizeGDB, including connections to many other online resources for genetic and genomic information. MaizeGDB is funded by the USDA/ARS and is publicly available for all to use at http://www.maizegdb.org.

An Update on the Comparative Maps of Maize and Rice in Gramene

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P31

Gramene (www.gramene.org) is a comparative mapping resource for rice and other cereal crops. The public resource leverages the rice genomic sequence to allow researchers working in maize and other monocots to apply knowledge derived from the study of the rice genome to corresponding syntenic regions in their species of interest. We present maize maps recently added to Gramene and the updated results of our maize-rice comparison.

The maize-rice comparison makes use of publicly generated data sources including the Finger Print Contig (FPC) physical map of maize available from the Arizona Genomic Institute and the MMP project, the public rice sequence draft available from the International Rice Genome Sequencing project (IRGSP), the rice genome pseudomolecules available from The Institute for Genome Research (TIGR), and the IBM2 Neighbors Map available from MaizeDB/MaizeGDB. In the analysis the rice genome acts as a scaffold to align maize sequences. The sequence similarities are then used as the foundation for correspondences between maps of maize and rice. The comparative maps and their correspondences are available at Gramene as graphical displays and in a downloadable tabular format. A user may enter a comparative map display using several methods, including a map study, a feature found on a map, or the "Matrix" view, a table that contains pairwise comparisons of the number of correspondences between two map studies in Gramene.

Cell Biology Posters

Study of the High Protein Trait of Maize Using the In Vitro Kernel Culture Model System

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Monsanto Company

P32

Protein production in maize kernels is a complex trait that responds to changes in source nitrogen nutrition, and few research tools are available to study this challenging kernel quality trait. A method for in vitro culture of individual kernels has been developed by B.G. Gengenbach (Planta 1977; 134:91-93) and others as a useful technique to study kernel

development. Kernels cultured in vitro can develop in a manner that is similar to kernels cultured in vivo. The method has been used successfully to study the effects of alterations of kernel nutrition on kernel metabolism as well as other aspects of kernel development. Studies were done to evaluate the technique of in vitro kernel culture as a research tool for understanding how kernel protein levels are controlled in maize. In this application, the quality, quantity and timing of nitrogen nutrition can be manipulated in order to study its effects on kernel protein synthesis. Both low and high protein genotypes were grown in vitro and source nutrition was altered in an effort to examine how genotypes that vary in kernel protein levels in the field may respond to changes in nutrient composition and quantity. Dry matter, nitrogen, carbon, and free amino acids were measured to profile the development of kernels cultured in vitro.

Interaction of the Plant Glycine-Rich RNA Binding Protein MA16 with a Novel Nucleolar DEAD Box RNA Helicase Protein from Zea mays

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P33

The maize RNA-binding MA16 protein is a developmentally and environmentally regulated nucleolar protein that interacts with RNAs through complex association with several proteins. By using yeast two-hybrid screening, we identified a DEAD-box RNA helicase protein from Zea mays that interacted with MA16, which we named ZmDRH1. The sequence of ZmDRH1 include the eight RNA helicase motifs and two glycine-rich regions with RGG boxes at the N- and C- terminus of the protein. Both, Ma16 and ZmDRH1 were located in the nucleus and nucleolus and analysis of the sequence determinants for their cellular localization revealed that the region containing the RGG motifs in both proteins was necessary for nuclear/nucleolar localization The two domains of MA16, the RNA recognition motif (RRM) and the RGG, were tested for molecular interaction with ZmDRH1. MA16 specifically interacted with ZmDRH1 through the RRM domain. A number of plant proteins and vertebrate p68/p72 RNA helicases showed evolutionary proximity to ZmDRH1. In addition, like p68, ZmDRH1 was able to interact with fibrillarin. Our data suggest that MA16, fibrillarin and ZmDRH1 may be part of a ribonucleoprotein complex involved in rRNA metabolism.

Developing Tools for the Study of Cellular Dynamics During Maize Development

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P34

The sequences of events accompanying cellular development of the maize plant have been poorly studied. In a bid to understand the complex cellular dynamics which occur during plant development, we have generated a range of transgenic marker lines useful for *in vivo* studies in maize. These lines contain different variants of green fluorescent protein (GFP) targeted to the cytoplasm, nuclear chromatin and cell wall. We have selected a number of independent lines suitable for the study of cellular dynamics in vegetative as well as in reproductive organs. These

lines have been introgressed into several inbred genetic backgrounds and currently, we are working towards the development of lines containing GFP variants targeted to all three cellular components.

Jasmonic Acid and Ethylene Modulate the Activation of Insect Defense Signaling Pathways in Maize

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Mississippi State University

P35

Induction of resistance to certain insect herbivores is generally regulated by a network of signal transduction pathways, which use ethylene and jasmonic acid (JA) as key signaling molecules. In this study, we used inhibitors of hormone synthesis and perception to determine if ethylene and JA regulate the accumulation of a 33-kD cysteine proteinase (Mir1-CP), a unique defense proteinase, that accumulates in response to caterpillar feeding in maize genotypes that are resistant to foliar feeding by fall armyworm (FAW, Spodoptera frugiperda) and other Lepidoptera. The accumulation of mRNAs encoding Mir1-CP (mir1) and lipoxygenase (LOX9) were also measured.

Whorls were treated with an inhibitor of ethylene perception [1-methyl cyclopropene (1-MCP)] or inhibitors of ethylene biosynthesis [2-aminoethoxyvinyl-glycine (AVG) or (CoCl2)]. FAW larvae fed on treated whorls for 4, 8, 24 and 48 hours and leave damage, larval weights, protein and mRNA accumulation were determined. Blocking ethylene perception and synthesis decreased the accumulation of Mir1-CP, and increased leaf damage and the weight of FAW larvae reared on the treated plants. However, no changes in mir1 accumulation were detected by conventional RT-PCR.

To determine if JA altered the defense response, plants were treated with ibuprofen, JA or JA and ibuprofen. Ibuprofen inhibits lipoxygenase (LOX) the first enzyme in the biosynthetic pathway of JA. Bioassays results showed that there was more FAW larval feeding in ibuprofen-treated plants than in water-treated controls as measured by leaf weight before and after feeding indicating loss in resistance. There was no notable feeding on the JA-treated plants implicating JA in the defense response. Semi-quantitative RT-PCR using gene-specific primers was performed to measure expression of mir1 and the JA responsive gene LOX9. To insure that no false positive PCR fragments would be generated from pseudogenes in contaminating genomic DNA, primer sequences were designed to span intron regions, when genomic sequence data was available. Semi-quantitative RT-PCR showed a significant increase in mir1 and LOX9 mRNA transcripts. Expression of 18S rRNA was constant in all treatments. These preliminary results suggest that JA and ethylene may act together to regulate the maize insect defense system.

Novel Regulation of Anthocyanin Pigmentation by Light

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The Ohio State University

P36

The induction of the flavonoid biosynthetic pathway by light is mediated by the increased expression of the regulators. Therefore, constitutive expression of the regulators should not alter the accumulation of anthocyanins in response to light. However, anthocyanin pigmented maize Black Mexican Sweet cells expressing the MYB and HLH regulators, C1 and R, from a light insensitive 35S CaMV promoter (35S::C1+R), showed visual darkening when exposed to high light. Interestingly, there was no increase in steady state mRNA levels for CHS (c2 gene), DFR

(a1 gene) or F3H (f3h gene), or in the quantitative or qualitative accumulation of the anthocyanins. In vivo reflectance measurements (CIELab values) showed an unexpected 'yellowing' of the light grown calli, as compared to the dark grown cells. A change in vacuolar morphology was observed in light grown 35S::C1+R calli. These results are indicative of an additional level of control of the pigmentation properties of the anthocyanins. The physiological and cellular effects of light on 35S::C1+R maize BMS cells are currently under investigation and will be discussed.

Regulation of the Expression of TOR and S6rp Kinase in Maize (Zea mays L.)

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P37

TOR (target of rapamycin) and the S6Ks (S6 ribosomal protein kinases) enzymes are members of a superfamily of serine/threonine protein kinases that have been identified as the principal regulators of cell growth in response to nutrients and insulin/growth factors. In maize tissues, insulin and a maize endogenous insulin-like growth factor (ZmIGF), stimulate a signal transduction pathway that regulates TOR and S6K. The objectives of this work is cloning the S6K and TOR cDNAs from maize and analyze their expression patterns at different developmental stages of maize embryos, as well as to investigate the effect of phosphatidic acid in regulating the activity of these enzymes, since this compound is known to induce the activation of both kinases in other eukaryotic systems. The experimental approach used in this work was the PCR and RT-PCR techniques, using a maize cDNA library or mRNA from maize embryonic axes as templates. By these approaches we cloned the orthologous cDNA for TOR and S6K from maize. The expression patterns of these cDNAs, evaluated by semi-quantitative RT-PCR, did not correspond to the protein content patterns. Whereas the mRNA level increases during germination, the protein content remains almost at the same level, although the protein activity increases during germination. Further, alignment of the maize TOR and S6K cDNAs with their animal counterparts showed high degree of identity among them, as well as maintenance of the corresponding regulatory domains in both enzymes. All the above data strongly suggest that the TOR and S6K orthologous in maize are implicated in the insulin/ZmIGF-induced signal transduction pathway like in mammals.

Comparative Proteomics of Mesophyll and Bundle Sheath Plastid Differentiation in Maize Leaves

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Cornell University

P38

Plastids are essential organelles present in every plant cell and are responsible for the synthesis of key molecules. Remarkably, plastids differentiate into different forms, depending of their localization in the plant. The molecular mechanisms that govern this differentiation remain largely unknown.

We have initiated a study of plastid differentiation during maize leaf development. In maize leaves, the photosynthetic function of plastids is distributed between plastids in two distinct cell types: the bundle sheath (BS) and the mesophyll (M) cells. It is known that plastids in the two cell types each accumulate a distinct set of photosynthetic enzymes enabling them to cooperate in carbon fixation. A developmental gradient exists within the maize leaf with incompletely differentiated plastids located at the base, and C4 type plastids located at the tip. The process

of differentiation and the functional specialization of the BS and M plastids in pathways other than primary carbon fixation are largely unknown.
We have developed a robust protocol for purification of BS and M plastids and their proteome from young maize leaves. Based on the abundance of specific markers for BS (e.g. Rubisco) and M (e.g. PPDK) chloroplasts, we estimate that the cross-contaminations of the purified BS plastid proteome and M plastid proteome are less than 10%. Several sets of high-resolution 2-Dimensional Electrophoresis (2-DE) maps of the two soluble plastid proteomes have been created and compared by image analysis. Proteins were identified using both Matrix-Assisted-Laser-Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and on-line LC-Electrospray Tandem Mass Spectrometry (LC-ESI-MS/MS). A preliminary statistical analysis of the differential expression of about 100 different proteins has been performed.
These data confirm the well-known preferential localization of C4 metabolism and carbon transport enzymes in the BS fraction. In addition, we found that starch and sulfur metabolism related enzymes are preferentially expressed in the BS fraction. Several anti-oxidative stress proteins were preferentially accumulating in M plastid; this is most likely related to the high, light-driven, photosynthetic electron transport rates in M thylakoids. Statistical analysis of these expression data is in progress and will be presented. The proteomics data will become available via our Plastid Proteome DataBase (PPDB) (http://cbsu.tc.cornell.edu/vanwijk/).
We have now also begun employing non-gel based tools for comparison of the BS and M protein expression profiles, using the cleavable Isotope-Coded Affinity Tags (ICAT) technique.

Root Cap-Quiescent Center: A Never Ending Dialog

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P39

The root cap (RC) a difference to other tissues, renewes in response to internal and external signals; in addition it has the ability to perceive and respond to environmental stimuli as gravity, light, humidity, etc. RC cells start their life as RC initials cells and they change their characteristics as they move in the RC, finally they leave the RC to become Border cells (BC) which control the cap meristem activity (1) For this to happen, it is necessary the participation of a neighbour tissue, the Quiescent center (QC) which supplies cells to reform the RC and RC meristem once the RC is naturally or surgically excised. Hormones control many aspects of growth and development in plants, moreover the interactions between plant growth hormones are important events in the regulation of plant development. The aim of this work is to dissect the hormonal elements of croos talk between auxin and ethylene to better undestand the so important functions of RC. In the lab we have characterized positional and structural RC markers which allow us to follow the RC cell development (2). Using these tools we studied in maize intact roots, the effect of stimulating cell divition in QC cells and how this affects gene activity in the RC. We treated maize root with the polar auxin transport inhibitor naphthylphthalamic acid (NPA), with indole acetic acid (IAA) in combination with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) or with a ethylene synthesis inhibitor 1-aminoethoxyvinilglycine (AVG). In some experiments we added to these drugs, 5-Bromo-2' -deoxy-uridine (BrdU) to study DNA synthesis as a parameter of cell division. As Border cell detachment can be experimentally induced (1), we studied the effect of auxin/ethylene levels in this physiologycal event.
Root treatment with NPA or IAA affect C123 expression, a positional marker in maize cap studied by in situ hybridization, AVG/IAA treatment induces QC activation and increases the

number of initial cells as reveled by BrdU incorporation, enlargement of root diameter and reduction in the number of border cell released, however, no change in the expression pattern of C123 was observed. The inhibition of polar auxin transport with NPA decreases C123 expression as was already mentioned and results in a remarkable increase in the number of border cells released; this effect is partially reverted by treatment with ACC. Both auxin and ethylene are endogenous regulators of root growth (3) however there is scarse information of these effects at root cap level. Our results suggest a clear interaction between auxins and ethylene in root cap development.

Establishment of Robust Maize Transformation Systems for the Public Sector

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P40

The long-term goal of this research is to establish a robust maize transformation system to enable the maize research community for future functional genomic research as well as crop improvement. Our specific objectives are:

1) Developing a routine Agrobacterium-mediated transformation system. We assessed transgenic maize events produced by particle bombardment or Agrobacterium-mediated transformation. Using Southern, Northern, real-time PCR, and real-time RT-PCR techniques, we compared transgene copy numbers and RNA expression levels in R1 and R2 generations of transgenic maize events generated using the these two gene delivery methods. The Agrobacterium-derived maize transformants have lower transgene copies, and higher and more stable gene expression than their bombardment-derived counterparts. We continue to optimize the transformation parameters to enhance the overall efficiency.

2) Enhancing transgene integration and expression. We are evaluating the effects of over-expressing the Arabidopsis histone H2A-1 protein (HTA1) in transgenic maize on re-transformation efficiency. Preliminary data suggest that the average efficiency of re-transformation in the H2A-1 expressing maize lines was higher than that of the control lines. We are also investigating the effects of maize chromatin genes on transgene integration and transformation frequency. A number of maize chromatin genes that either increase or decrease maize transformation efficiency were identified and tested in transgenic maize (see poster by McGill et al.). Additionally, we have been investigating the fundamental roles of transcriptional and post-transcriptional gene silencing on transformation and transgene expression. We have conducted a series of experiments in model systems of tobacco cell lines and Arabidopsis thaliana plants. The results of these experiments are being applied to maize. The results suggest that the transcriptional state of the incoming DNA can increase subsequent silencing, and that the relative role of PTGS is dependent on the cell division rate. In fact, the increase in transcription initiation potentiated by MARs can increase PTGS induction. Thus, transcriptional gains facilitated by MARs may need to be offset by additional transformation strategies to minimize PTGS.

3) Investigating germline transformation protocols. We are investigating tissue culture-independent maize transformation protocols such as meristem transformation and female gametophyte transformation.

4) Exploring inbred line transformation. We are conducting research to improve transformation efficiency (both biolistic and Agrobacterium-mediated methods) on a number of public inbred lines.
5) Facilitate transfer of maize transformation protocols to the public sector. We organized a Maize Transformation Workshop (NSF funded) at Madison, WI, from March 10-13, 2003. Both Biolistic-mediated and Agrobacterium-mediated maize transformation services are available at the Plant Transformation Facility of Iowa State University (http://www.agron.iastate.edu/ptf/Web/mainframe.htm).

Cytogenetics Posters

Comparative Cytogenetic Map of Two Maize Inbreds: Mo17 and B73

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Pioneer Hi-Bred Int., a Du Pont Company

P41

A collection of various clustered repeats and "gene-rich" BAC clones were used as labeled probes in FISH experiments to map corn chromosomes. We identified a minimal set of repetitive sequences, which allows the identification each of the 10 chromosomes and their arms. Significant polymorphism was detected for the majority of clustered repeats in the number of hybridization sites, size, and distribution on chromosomes of these two corn inbred lines. Structural polymorphism of repeat clusters was confirmed by direct DNA sequence comparison of homologous segments from these two lines. These observations suggest that the corn genome contains multiple repeat tracts of significant length (some could be >1 Mbp) in each corn line that disrupt the linear pattern of the gene scaffold between maize genotypes.

Recombination Rate, EST Distribution and Gene Clustering along the Physical Structure of Maize Chromosomes

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P42

ESTs are dispersed more-or-less evenly along the genetic map of each chromosome (although there is a tendency for ESTs to clump more near centromeres). However, it is well-known that genetic map length does not correlate well with the physical structure of chromosomes. Using the distribution of high-resolution cytological markers of crossing over (recombination nodules, RNs) in maize, it is now possible to predict the physical location of genetically mapped markers with a high degree of accuracy. Here, we have estimated the physical position of 1195 genetically mapped EST markers on the ten pachytene chromosomes of maize. We compared the physical distribution of ESTs along each chromosome with the expectations based on 1000 random (uniform) distributions. In the uniform distribution, each 0.2 chromosome length interval has an equal probability of having an EST. There was a significant difference between the observed and expected distributions of ESTs for each chromosome and each chromosome arm. This is expected based on low frequency of genes in heterochromatic compared to euchromatic chromosome segments. However, when we examined the distal halves of each chromosome arm (that includes only euchromatin), there was also a significant difference between the observed and expected distributions, particularly for the long arms of the chromosomes. This result indicates that ESTs (and genes) tend to be clustered more than expected from a random distribution. We also found a positive relationship between EST and RN frequency (using 10% chromosome arm length intervals) for each chromosome. When the data from all the

chromosomes were pooled together, the regression between EST and RN frequency and location was significant (r2 = 0.59). These results support other work that indicates that most crossing over in plants occurs near or within genes. Interestingly, for most chromosomes, the most distal chromosome segments have fewer ESTs than expected based on the high distal recombination rate. It is not clear whether these telomeric segments of chromosomes actually lack ESTs or whether this is simply an EST sampling artifact.

Organization of Endoreduplicated Chromosomes in the Endosperm

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P43

In the later stages of development of the triploid endosperm, the chromosomes undergo endoreduplication. Endoreduplication is believed to occur through a loss of the M and G1 phases of the cell cycle, which creates a cessation of cell division but a repetition of the S and G2 phases. Consequently, the normally triploid endosperm cell can reach a ploidy level up to 384x in some lines of maize. There have been many studies on endoreduplication that have investigated the genes involved, influence of hormones, timing, epigenetic control, and environmental influences. Nevertheless, the molecular mechanism is still poorly understood. We have undertaken a study to examine the structure of the endoreduplicated chromosomes. Previous cytological work has indicated that, although the DNA content per cell increases, the number of nucleoli and knobs remain the same. It has been suggested that only regions with transcribed genes are amplified. With the use of fluorescence in situ hybridization techniques, we have shown that the highly repetitive heterochromatic areas, as well as several actively transcribed genes, are successively replicated, which suggests that the entire genome follows the same trend. Further evidence shows that the multiple copies, after they have been replicated, stay associated throughout the length of the chromosomes, and that the DNA at the centromeric and knob regions are more tightly associated than the other regions of the chromosomes.

Characterization of the elongate1 Mutant in Maize

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IRD-CIMMYT

P44

Maize is extremely well suited for studying meiosis. Among the numerous maize meiotic mutants described over the years, we are particularly interested in the el1 (elongate 1) locus. The el1 mutant, which results in the production of a variable proportion of unreduced female gametes has several interesting features, including very unique defaults in chromosome condensation (which remain ‘uncoiled’ at meiosis), and, in contrast to most know meiotic mutants, a surprising level of fertility in spite of a severely abnormal meiotic progression. We present a cytological and molecular characterization of the el1 mutant. Our cytological data shows that el1 induces numerous defaults during both meiosis I and meiosis II. We used Mutator transposons to tag and clone el1. Two independent alleles were recovered from a targeted screen. The Mu-tagged locus encodes a protein of yet unclear function.

What is the Role of the Noncrossover Recombination Pathway in Meiosis?

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P45

We are interested in the role of recombination in meiosis. From previous work in maize, we know that localization of foci containing the recombination protein RAD51 is coincident with the time of pairing, and that lack of RAD51 foci correlates with non-homologous pairing (Pawlowski et al, Science 303:89-92, 2004). We also know that in maize there are many more RAD51 foci (at least 500) than are required for the 23 or so crossovers observed in each individual meiocyte. Taken together, this suggests a role for recombination enzymes in the homology search. A meiotic recombination event is initiated by a double strand break (DSB), followed by single strand invasion and the formation of a double holiday junction (DHJ). Currently, it is thought that there are two separate pathways downstream of DSBs and RAD51 function that lead to resolution of DHJs: a pathway that leads to crossovers (genetic 'recombination' that allows us to make genetic maps), and a noncrossover pathway. The role of the noncrossover pathway in meiosis is not clear, but it is possible that DSBs are first used for homology searching, and then most are repaired by the noncrossover pathway and only the few DHJs needed for chiasmata are resolved by the crossover pathway. It is not known how the choice between the noncrossover and crossover pathway is made; however, it is known in mouse that noncrossovers occur before noncrossovers and we speculate that an interference mechanism is involved, as crossovers display interference, while noncrossovers do not. In order to understand the role of the noncrossover pathway in meiosis, we have started a new project looking for mutants that disrupt this pathway. In yeast, a recQ helicase-like protein called sgs1 is implicated specifically in the noncrossover pathway. To find homologs of recQ in plants, we performed a phylogenic analysis of plant recQ-like proteins using a variety of inference methods. While we were looking for homologs in maize, we found 6 Arabidopsis homologs and decided to explore all of these. We have obtained T-DNA insertions in most of these genes, and are examining the mutant phenotypes for effects on meiosis. Here we present our strategy and current progress.

The Behavior of Abnormal Chromosome 10 in the Monosomic Condition

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P46

When the diploid maize chromosome complement contains at least one copy of abnormal chromosome 10 (Ab10), three phenomena are observed: an increased rate of recombination, neocentromere activity, and meiotic drive (preferential segregation). However, it is not known whether Ab10 must be present in a diploid fashion (which is to say, it is not known if Ab10 needs a pairing partner to be active). The r-X1 deficiency is an X-ray induced deficiency that includes the R locus on chromosome 10. A high rate of non-disjunction occurs in embryo sacs that contain the r-X1 deficiency producing monosomics at a rate of ~10%. To find out how Ab10 behaves in the unpaired condition, crosses are being carried out to produce plants that are monosomic for Ab10, N10 (the normal variant of chromosome 10), and smd3-Ab10 (Ab10 carrying the suppressor of meiotic drive 3 mutation) chromosomes. Each plant type will be crossed to N10/N10 plants, and ovule abortion rates will be determined for each genotype (N10, Ab10, and smd3-Ab10). The N10 and smd3-Ab10 values will be used as controls for comparison to Ab10 segregation. It is important to note that this project is designed to

determine the effect of monosomy of Ab10 on meiotic drive. Whether Ab10 monosomy affects recombination rates and/or neocentromere activity are not directly tested by this method, though interesting hypotheses concerning recombination rates and neocentromeric activity may emerge from the resulting data.

Meiotic Recombination and Stress in Maize

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P47

The effects of water stress and defoliation on meiotic recombination have been investigated for genotypes B73/Mo17 and Mo17/H99. F1 plants were exposed to either stress or non-stress treatments. For each genotype, 6 populations were created by backcrossing 6 F1 plants (3 F1 plants from each treatment) as males to B73 or H99, respectively. 93 seedlings of each population were genotyped at microsatellite loci to create genetic maps for chromosomes 1 and 10. For B73/Mo17, the maps of chromosomes 1 and 10 were larger for water-stressed plants (172 vs 149 cM for ch. 1; 83 vs 72 cM for ch. 10). In the B73/Mo17 populations, crossovers were not detected in 19 and 44% of the gametes for chromosomes 1 and 10, respectively. For Mo17/H99, the maps of chromosomes 1 and 10 were also larger for water-stressed plants (177 vs 161 cM for ch. 1; 84 vs 65 cM for ch. 10). In the Mo17/H99 populations, crossovers were not detected in 17 and 48% of the gametes for chromosomes 1 and 10, respectively. In both genotypes, 1 interval of chromosome 10 exhibited significantly more recombination. Defoliation of seedlings did not affect recombination.

Maize Centromeres: Organization and Functional Adaptation in the Genetic Background of Oat

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P48

Centromeric DNA sequences in multicellular eukaryotes are often highly repetitive and not unique to a specific centromere or to centromeres at all. Thus, it is a major challenge to study the fine structure of individual plant centromeres. We used a DNA fiber-fluorescence in situ hybridization approach to study individual maize centromeres using oat-maize chromosome addition lines. The maize centromere-specific satellite repeat CentC in the addition lines allowed us to delineate the size and organization of centromeric DNA of individual maize chromosomes. We demonstrate that the cores of maize centromeres contain mainly CentC arrays and clusters of a centromere-specific retrotransposon, CRM. CentC and CRM sequences are highly intermingled. The amount of CentC/CRM sequence varies from ~300 to >2,800 kilobases among different centromeres. The association of CentC and CRM with centromeric histone H3 (CENH3) was visualized by a sequential detection procedure on stretched centromeres. The analysis revealed that CENH3 is always associated with CentC and CRM but that not all CentC or CRM sequences are associated with CENH3. We further demonstrate that in the chromosomal addition lines where two CenH3 genes were present, one from oat and one from maize, the oat CENH3 was consistently incorporated by the maize centromeres.

Initiation of Meiosis in Maize by ameiotic1

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P49

In maize, as in other multicellular organisms, initiation of meiosis is preceded by a developmental pathway that assigns identity to germline cells and leads to the formation of meiocytes. Subsequently, meiocytes undergo a programmed switch from the somatic to the meiotic cell cycle. In maize, this switch is regulated by the ameiotic1 (am1) gene. The first mutant allele of am1, am1-1, was discovered by Marcus Rhodes in 1956. In most mutant alleles of am1, meiocytes undergo mitosis instead of meiosis or arrest in a pre-division interphase. The mutant meiocytes, although normally developed, show mitotic chromosome structure and condensation, and have a mitotic cytoskeleton. We now demonstrate that the am1 gene also regulates installation of the meiotic recombination machinery, as meiocytes in am1 mutants lack chromosomal foci of the recombination protein RAD51 involved in repairing meiotic double-strand breaks. In contrast to the other am1 alleles, in the am1-praI allele meiocytes enter meiosis but arrest in early meiotic prophase. Our data indicate that the arrest occurs at the leptotene - zygotene transition, as suggested by incomplete telomere bouquet formation and the presence of double-strand breaks (which are normally formed in leptotene) while RAD51 fails to load onto chromosomes (which normally happens in zygotene). Interestingly, chromosomes in some am1-praI meiocytes continue condensation and assume a pachytene-, or sometimes even, a diplotene-like morphology, suggesting that progression of chromosome condensation has become uncoupled from the overall arrest of meiosis. Analyses of mutant phenotypes of the various am1 alleles suggest that the AM1 protein functions during pre-meiotic interphase, most likely to change the identity of the S phase from mitotic to meiotic. We cloned the am1 gene using a new transposon-tagged allele. It encodes a novel protein with unknown biochemical function. Interestingly, the am1 transcript is constitutively expressed in all plant parts and not limited to meiocytes. This suggests that during the mitosis-to-meiosis switch, the AM1 protein interacts with other proteins that are expressed in a cell cycle-dependent manner. The hypothetical AM1 protein contains two predicted coiled-coil domains, frequently associated with protein-protein interactions. We are now testing the hypothesis that AM1 interacts directly with PCNA, a multi-functional DNA 'sliding clamp' protein that is involved in the progression through the S phase and is known to interact with cyclins and chromatin remodeling proteins.

Integrating Genetic Linkage Maps with Pachytene Chromosome Structure in Maize

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P50

Integrating genetic linkage maps with chromosome structure has been an important objective ever since it was demonstrated that genes occur in a fixed order on chromosomes. Linkage maps are defined by the percentage of recombination between markers [as expressed in centiMorgans (cM)] and reveal the linear order of markers. However, they do not contain information on the actual physical distance between markers, whether that distance is expressed as a cytological length (positions on chromosomes) or as a physical length (number

of DNA base pairs). This is because crossing over is not evenly distributed along chromosomes. Crossing over is suppressed in heterochromatin and centromeres, and crossing over is variable even in euchromatin where most crossing over occurs. As a result, linkage maps cannot be simply overlaid on chromosomes to determine the physical position of genes. One way to integrate linkage maps with chromosome structure is to utilize high-resolution cytological markers of crossing over, such as recombination nodules (RNs). RNs are proteinaceous, multi-component, ellipsoids approximately 100 nm in diameter, which are found in the central region of synaptonemal complexes (SCs) between homologous chromosomes (bivalents) at pachytene. Evidence that RNs mark crossover sites include the close correspondence between the frequency and distribution of RNs compared to chiasmata, the presence of an essential crossover protein (MLH1p) in RNs, and the presence of MLH1p/RNs at chiasma sites. Because RNs can be observed only by electron microscopy of SCs in elongate pachytene bivalents, RNs represent the highest resolution markers available for determining the chromosomal location of crossing over. Each RN represents one crossover between two homologous non-sister chromatids, which yields two recombinant and two parental chromosomes that is, by definition, equivalent to 50 cM on a linkage map. On this basis, the frequency of RNs can be converted to cM and used to prepare a detailed map of recombination along the physical length of each of the ten pachytene chromosomes/SCs in maize. Because RN maps relate the amount of recombination to cytological position along pachytene chromosomes and linkage maps report the amount of recombination relative to genes or other markers, it is now possible to combine these two approaches to directly relate genetically-mapped markers to cytological position. We have used this procedure to predict the physical position of genetically mapped core bin markers on each of the ten chromosomes of maize. We tested our predictions for chromosome 9 using seven genetically-mapped, single-copy markers that were independently mapped on pachytene chromosomes using in situ hybridization. The correlation between the predicted and observed locations was very strong ($r2 = 0.996$), indicating a virtual 1:1 correspondence. Thus, this new, high-resolution, cytogenetic map enables one to predict the chromosomal location of any genetically-mapped marker in maize with a high degree of accuracy. This work was supported by the National Science Foundation (MCB-9728673 to SMS, MCB-0314644 to LKA, and DBI-9813365 to ZC and LH), the Consortium for Plant Biotechnology Research, Inc. (DOE OR22072-102) and the Florida State University Research Foundation (to HB), and Eiselenstiftung, Ulm, Germany (to GW).

Localization of Large DNA Fragments Transferred into Maize Chromosomes by Agrobacterium Infection

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University of Missouri – Columbia

P51

Large insert cloning vectors make it possible to examine large genes and gene complexes, to identify long-range cis-regulatory elements, to create transgenic models that more faithfully reproduce the functional aspects of endogenous loci, and to complement known genetic mutations. In plants, there have been a number of reports studying the transfer of large DNA fragments using Agrobacterium tumefaciens vectors. Here we report about the use of the BIBAC technology to transfer 50kb T-DNA into maize chromosomes, and its chromosomal localization by fluorescence in situ hybridization (FISH). A 35kb yeast genomic DNA was placed between the selection marker gene, bar, at the left T-DNA border, and the Cre recombinase gene, at the right T-DNA border: bar-yeast piece-cre. This plasmid, pJV21, was transferred from E. coli DH10B to Agro LBA4404 by electroporation, and then used in the transformation of HiII

immature embryos. Seventy-five transgenic lines carrying pJV21 developed into type-II calli and regenerated embryos growing on N6 media supplemented with the herbicide bialaphos. Southern blots confirmed the presence of the bar gene in all lines. To assay for Cre expression and transmission, maize embryos resulting from the crosses of the transgenic lines with the tester, were bombarded with plasmid pHK52, which carries a ubiquitin promoter fused to an antisensse GUS c-DNA, flanked by two loxP sites oriented in opposite directions (Srivastava and Ow, 1999). Plants carrying a functional Cre recombinase will invert the GUS gene to the sense orientation. As a result, blue sectors were found in transgenic embryos bombarded with pHK52. GUS expressing sectors were never observed in non-transgenic embryos. Embryos from the cross of J11-24 with non-transgenic HiII were stained for GUS activity 4 days after the bombardment with pHK52. Localization of the T-DNA insertion sites by FISH, using the 35kb yeast genomic DNA as probe, was obtained in interphase nuclei and in metaphase and pachytene chromosomes of the transgenic lines. Spreads from transgenic line J10-6 were hybridized simultaneously with the yeast-piece (FITC) and an 18S rRNA probe (Texas-Red). The T-DNA is located in the long arm of chromosome 6, which carries the rRNA genes in the short arm satellite. Some of the future applications of the BIBAC-FISH technology are the transfer to maize of full regulatory pathways, and the analysis of transgene organization and interaction at the cytological level. Moreover, in combination with the Cre-loxP system, it can be used to generate predictable chromosomal rearrangements in maize, as has been reported for tobacco and mice.

Chromosomal Localization of Transgenes in Maize by Fluorescence In Situ Hybridization

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P52

FISH is a sensitive method developed for the detection of genes or DNA sequences on chromosomes. A transgene complex with a 35 kb yeast genomic DNA tag has been introduced into maize by Agrobacterium-mediated transformation (see Vega et al. abstract). By using the 35 kb yeast genomic DNA as a probe, we have detected the transgenic complex linked to the yeast DNA in maize metaphase chromosomes. Here we report a system to identify the transgenic integration sites on maize chromosomes by combining the yeast probe with a multi-probe karyotyping mix (see Kato et al. abstract). We first karyotyped the HiIIA and HiIIB lines, the parents of the HiI hybrid used for gene transformation, by using a multi-probe mix (Kato et al. abstract). A biotin-labeled yeast probe was mixed with the karyotyping mix, and hybridized to the metaphase chromosome spread. The biotin-labeled probe was detected with horseradish-peroxidase conjugated streptavidin, and amplified with biotinyl tyramide followed with DTAF-conjugated streptavidin. To date, we have localized 30 transgenic integration sites on maize chromosomes by this method. The integration events seem to be unevenly distributed among the 10 maize chromosomes, with nine of the 30 integrations on chromosome 1, and eight on chromosome 7. Interestingly, all of the integrations on chromosome 7 are located near the knob. A southern analysis of these transgenic lines probed with the Bar gene sequence revealed different band sizes after digestion with XhoI, an enzyme that does not have a recognition site between the Bar gene and the border sequence of the T-DNA. This observation indicated that each transgenic event was independent, and eliminated the possibility that these integrations were in the same site. In addition to the eight integrations near the chromosome 7 knob region, we also detected 13 integrations in the distal region of other chromosomes. Because FISH on

metaphase chromosomes can only give an estimation of the location, it will be interesting to use other methods to determine whether there are any preferred insert sites.

Developmental Genetics Posters

Dissecting the Mechanisms of Sex Determination in Maize

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P53

The sex determination (SD) pathway of maize provides one of the best non-animal genetic systems to study the cell death and cell arrest processes. Our mission is to understand this pathway, including the associated tasselseed-mediated pistil cell death, silkless-mediated pistil cell protection, and gibberellin (GA)-mediated stamen cell arrest. Several mutations affecting sex determination in maize have been genetically characterized but the isolation of the corresponding genes such as silkless1, tasselseed1 and pistillate has remained elusive. In order to identify these genes, we are exploring diverse strategies that include transposon tagging, map-based cloning and whole genome subtractive hybridization approaches. In the other hand, we are identifying additional mutations in the SD pathway through genetic screenings using transposons and we have used a two-hybrid approach to discover interacting partners to Tasselseed2, the first SD gene cloned so far. The last approach has yielded two interacting partners designated TIP1 and TIP2. TIP1 encodes a second short-chain alcohol dehydrogenase/reductase (SDR) with extensive similarity to TS2, raising the hyphotesis that these proteins can work as a heterodimer.. Mapping of TIP1 in the maize IBM94 population has discarded the possibility that it corresponds to tasselseed1. A mutant allele of TIP1 carrying an Ac element in the coding sequence is currently being characterized as to the presence of SD mutant phenotypes. TIP2 encodes a type-5 protein phosphatase (PP5) with extensive similarity to the human PP5, a protein that has several and important roles in signal transduction such as cell cycle regulation and signaling by nuclear receptors.

Xochiquetzal (XOC), an Arabinogalactan Protein Essential for Female Gametogenesis in Arabidopsis thaliana

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P54

Intercellular communication plays a crucial role in plant development and it is generally believed that cell fate is largely determined by positional information. Several key regulatory proteins have been shown to play important functions in cell signaling and recognition. Arabinogalactan proteins (or AGPs) are an abundant class of highly glycosylated hydroxyproline-rich glycoproteins implicated in many developmental programs including vascular differentiation, floral organogenesis, pollen tube growth and somatic embryogenesis. Classical AGPs contain a domain responsible for anchoring the protein to glucosylphospatidyl-inositol (GPI). Subsequently, processing of C-terminal domain allows release from the membrane. Recently, an non-classical AGP was shown to be involved in root regeneration and seed germination (K. Roberts and A Van Hegel 2003, Plant Journal 36:256-70); however, the specific function of a classical AGP has yet to be determined. The haploid female gametophyte (embryo sac) of Arabidopsis is composed of 7 cells: two synergids, the egg cell, three antipodals and a binucleated central cell. Using a collection of enhancer detector lines generated in our laboratory with the system implemented by Sundaresan et al 1995, we identified

XOCHIQUETZAL (XOC; named after the floral goddess of the Aztecs), an enhancer detector line showing a specific pattern of expression in the female gametophyte and the young embryo, but with no obvious mutant phenotype. TAIL-PCR analysis revealed that this line has two enhancer detector elements inserted in the regulatory region of a gene encoding for an arabinogalactan protein. XOC is expressed in female reproductive tissues throughout ovule development and early embryogenesis, but also in vegetative tissues such as stems and seedlings. To determine the role of XOC during female reproductive development, we used the pFGC5941 dsRNA vector (kindly provided by C. di Napoli and R. Jorgensen, U. of Arizona) we cloned a palyndromic portion of 5' UTR and the first exon and silenced XOC by RNA interference (RNAi). Transformants showed female gametophytes arrested after the differentiation of the functional megaspore but showing normal ovule morphology. Our results show that XOC plays an essential role at the onset of the female haploid phase in Arabidopsis.

Characterization of Maize rop2 Mutant Pollen Suggests Multiple Roles for the ROP2 GTPase in Pollen Tube Development

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P55

Successful reproduction in higher plants requires communication between the male and female gametophytes to coordinate pollen tube guidance and fertilization. However, the molecular and developmental mechanisms involved in many of the stages of male gametophyte development (e.g., in fertilization) are largely unknown. We have previously demonstrated that mutation of the maize rop2 gene, encoding a ROP GTPase, compromises the competitive ability of pollen. When used alone, rop2 mutant pollen produces fully fertilized ears. However, when placed in competition with wild-type pollen, rop2 mutant pollen fertilizes the female gametophyte at a significantly lower frequency than the wild-type. Previous work on the most likely rop2 ortholog in Arabidopsis, AtROP1, indicates that Rop can control pollen tube growth and morphology in vitro (Li et. al, 1999; Kost et. al, 1999), but these effects have not yet been shown in vivo. Using our strongest rop2 mutant allele, rop2-m1, we have been investigating the effects of the rop2 mutation on pollen germination, pollen tube growth and guidance, and fertilization. In dual pollination experiments in which silks are pollinated with rop2 mutant pollen and then 'chased' with wild-type pollen at different time points, mutant pollen is out-competed by wild-type pollen at the early time points, suggesting that mutant pollen is delayed in its ability to fertilize ovules. However, measurement of pollen tube germination and growth rate in vitro indicated that both are unaffected by the rop2 mutation. In vivo growth patterns of pollen in the silk, observed using a fluorescent stain for callose in the pollen tube cell wall, also did not appear significantly different between wild-type and rop2 mutant pollen. Analysis of populations from the apex and base of ears generated from outcrosses in which a rop2 mutant heterozygote was used as a male parent revealed no significant differences in the proportion of mutant progeny. This indicates that the competitive defect in rop2 gametophytes is not exacerbated by silk length, suggesting that the rop2 mutation does not significantly affect pollen tube growth rate in vivo.

Current investigations are focused on the growth patterns of pollen tubes at the silk base and near the ovule. Preliminary data suggest that mutant and wild-type pollen tubes arrive at the silk base at the same time, supporting our inference of similar growth rates in the silk. We are now testing the hypothesis that at least one rop2 pollen defect occurs at a final stage of male gametophyte development: either pollen tube guidance to the egg sac, or fertilization of the egg sac. Staining experiments comparing the time courses of pollen tube arrival at the micropyle and embryo development after fertilization will be presented.

Mapping of the Allele pt-McClintock at a Distinct Locus From Pt1*

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P56

In an effort to more fully understand maize floral organ development, we are studying a recessive ear mutation, polytypic ear (pt*-McClintock). The ears of this floral development mutant have extra silks and kernel mis-rowing, due to the failure of the second floret to abort during development. Using PCR amplification of microsatellite markers, we have identified several linked loci that place pt* on the long arm of chromosome 5. These preliminary mapping data suggest that pt*-McClintock describes a distinct locus from Pt1, which maps to chromosome 6. Other genes with similar phenotypes - such as zag1 and silky1 - are members of the MADS-box gene family, whose members code for a conserved DNA binding region and some of which have roles in angiosperm floral organ development. A possible candidate gene for pt* is zag5, a class-C floral development gene that maps to the same chromosome arm. We will do cosegregation analysis to explore this possibility.

Analysis of Mu-Tagged Empty Pericarp Mutants from the UniformMu Maize Population

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P57

Empty pericarp mutants represent a large class of relatively unexplored mutants. In mature seeds of these mutants, the pericarp appears collapsed and surrounds an area substantially devoid of endosperm or embryo tissue. In the few cases studied, the endosperm and embryo undergo early development, but then development arrests and tissues are resorbed (Fu et al. 2002. Plant Cell 14:3119; this study). Whether this is a universal feature of empty pericarp mutants remains to be determined. This class of mutants is likely to define important genes involved in a broad range of developmental and metabolic processes. They are also of potential interest because an empty pericarp kernel is in essence a seedless fruit. We have undertaken a survey of the developmental and cellular basis for the phenotypic defects in this class of mutants to gain a fuller appreciation of processes that may be disrupted and to identify genes of potential interest. Noteworthy are a mutant that does not appear to accumulate starch grains, and another that appears defective in the differentiation of the basal endosperm transfer layer. A molecular analysis using the Mu-TAIL procedure to identify the genetic lesions responsible for several of these mutants is underway.

Maize CLAVATA3-functional Ortholog

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P58

Maintenance of the shoot apical meristem (SAM) involves the coordination of cells recruited to organogenesis and the self-renewal of the stem cell population. In Arabidopsis, a trimeric CLAVATA (CLV) receptor complex functions to regulate the size of the stem cell population. The CLV receptor complex consists of a transmembrane LRR (leucine-rich repeat) kinase, CLV1; a transmembrane LRR protein, CLV2; and a small, secreted polypeptide, CLV3. All recessive loss-of-function CLV mutants form enlarged SAMs that are often fasciated. In

monocot species, the CLV pathway also appears to be functionally conserved. The fasciated ear2 (fea2) mutant in maize was found to encode an LRR protein with high similarity to the CLV2 gene in Arabidopsis. We have identified another member of the CLV receptor complex in maize, CLV3-like. Both genomic and predicted protein sequence structure of the maize CLV3-like gene are similar to the Arabidopsis gene. The maize sequence was mapped to chromosome 2.04 that is syntenic to chromosome 4 in rice where a predicted rice ortholog was identified. The low levels of the maize CLV3-like RNA were detected in predominantly meristematic tissues and in situs RNA hybridizations show resticted expression in developing ear florets. Ectopic expression of the maize CLV3-like gene in Arabidopsis showed strongly attenuated plant growth similar to that produced by ectopically expressed Arabidopsis CLV3. The attenuated plant growth phenotype in Arabidopsis is dependent on a functional CLV1 gene product. Current efforts are underway to identify deletion mutant lines to unequivocally associate mutant phenotype with gene function in maize. Taken together, these data suggest that the maize CLV3-like gene is functionally orthologous to the Arabidopsis CLV3.

Genetic and Molecular Analysis of the Wavy Auricle in Blade (wab1) and Milkweed Pod (mwp) Mutants of Maize

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P59

Leaves of maize are highly patterned structures that originate from 100-200 cells recruited in the shoot apical meristem to form a leaf primordium. From the base to the apex, maize leaves consist of two domains, the sheath and the blade, separated by an adaxial epidermal fringe, the ligule, and its associated auricle. We are working toward the map-based cloning of Wab1, a dominant mutation that disrupts this basic pattern and conditions proximal-distal tissue transformations in the leaf in a manner independent of ectopic knox function (Hay and Hake, accepted pending revisison to Plant Phys, 2004).

We have also focused our attention on the milkweed pod mutant, first described by Dr. Oliver Nelson, which displays tissue outgrowths resembling ectopic margins on the husks, a phenotype that may be attributed to a putative shift in leaf dorsoventrality. Our progress on the phenotypic characterization and the map-based cloning of mwp and Wab1 will be presented.

Pangloss Genes are Required for the Asymmetric Divisions of Subsidiary Mother Cells in Maize Stomata

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P60

The asymmetric cell divisions leading to the formation of stomata are among the most important in the development of the maize leaf. It is through stomata that plants are able to perform both gas exchange and transpiration. In maize, stomata are composed of four cells: central guard cell pair, which forms the stomatal pore, and a flanking pair of triangular subsidiary cells.

We have isolated mutations in two genes responsible for the asymmetric division of subsidiary mother cells (SMCs) to form subsidiary cells. In both pangloss1 and pangloss2 mutants, SMCs fail to polarize correctly, and the resulting subsidiary cells often protrude into neighboring cell files and frequently fail to specify as subsidiary cells. Cytoskeletal analysis reveals abnormalities in the organization of both actin and microtubules. Double mutant analysis suggests Pan1 and Pan2 act in separate, possibly parallel, pathways leading to the formation of subsidiary cells.

Individuals in the brick family of mutants show defects in subsidiary cell division very similar to that of pangloss mutants. Preliminary analysis of pan;brk double mutants suggest Pangloss and Brick act in separate genetic pathways to promote SMC polarization and division.

Evidence of Programmed Cell Death and its Possible Role in the Functional Activation of Placento-Chalazal Layer in the Pedicel Tissue of Developing Maize Caryopsis through Maternal-Filial Interaction

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P61

Cumulative evidence based on cellular and ultracellular level studies have shown programmed cell death (PCD) in placento-chalazal (PC) cell layers of maternal pedicel tissue in developing caryopses of normal seed, Mn1, and in the invertase-deficient miniature (mn1) seed mutant in maize. PCD was evidenced by loss of nuclei and all sub-cellular membranous organizations in many PC cell layers. The “empty” cells, however, remained in situ providing a critical physical continuity between the mother plant and the developing seed throughout the seed development. The TUNEL stain that is diagnostic of apoptoptic-like PCD identified spatially and temporally two distinctive sub-domains, which coincided with the previously described nucellar and integumental PC layers. The early wave of PCD, specific to the nucellar PC (directly underneath the basal endosperm), was TUNEL-negative and was seen in only the fertilized caryopses, indicating that the signaling for enucleation in these maternal cells originated in the zygotic tissues. In fact, the initiation of enucleation coincided with endosperm cellularization and was rapidly and coordinately completed in several files of the PC layer prior to the beginning of the major storage phase in endosperm. Cell shape in the PC layers also appeared to be influenced by the genotype of filial endosperm. Specifically, the enucleated PC cells in the Mn1 kernels were distinctively compact and oblong, whereas , the mn1 cells were irregular in shape and were slightly larger than the Mn1 cells. The later wave of PCD was restricted to the integumental PC underneath the enucleated cells of the nucellar PC, and was TUNEL-positive in both genotypes. In some cells, the TUNEL staining pattern appeared as foci concentrated around the periphery of cell membrane. These foci were DAPI-positive, an indication that they were due to membrane-bound apoptotic-like fragmented DNA. At ultracellular level, we observed a progressive condensation of the nuclear DNA which underwent fragmentation, formed apoptotic-like bodies and, ultimately, lost. In addition, there were progressive losses of plasmodesmata (at 12 DAP, there might be only the vestigial PDs as these cells have no protoplasm), mitochondria, vacuoles and all other membraneous oranizations from these cells. The two subdomains of PC layers were also distinguishable by UV-induced autofluorescence indicating the presence of unique sets of cell wall associated phenolics and flavonoids. Based on collective evidence we suggest that the early wave of PCD, presumably of osmolytic etiology, is an adaptive strategy to provide a clear passage for rapid transport of nuitrients and water to a developing sink tissue. The second wave of PCD may be senescent-related; the flavonoids may have a protective role against microbes that may be transported from the maternal tissue. The progressive loss of plasmodesmata implies that the short distance post-phloem transport may be independent of the symplastic passage. We suggest that the control of transport from the maternal cells may depend on the collective forces of turgor and metabolic sensing in the basal endosperm cells.

Microarray Analysis of the Branched Silkless Mutant of Maize and the Frizzy Panicle Mutant of Rice

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P62

The spikelet is a compact floral branch that consists of sterile bract leaves called glumes that enclosed a variable number of florets. In branched silkless1 (bd1) mutants the spikelet meristem takes on the identity of a branch meristem and produces branches instead of florets. We cloned the bd1 gene and showed that it encodes an ERF transcription factor. Recently, several groups working with rice have shown that the frizzy panicle (fzp) gene represents the bd1 orthologue of rice. In order to identify and compare the target genes of bd1 and fzp in maize and rice, micro array analysis was done. A full genome rice oligo chip was probed with cDNA made from fzp mutants as well as bd1 mutants. Conversely, a maize cDNA chip was probed with cDNA made from bd1 mutants as well as fzp mutants. The success of the cross species hybridization experiments will be discussed along with the resulting gene list generated by each experiment. True target genes were distinguished from indirect targets based on the presence of the BD1/FZP binding sites in their promoters.

Identification of Genes Associated with Root Architecture Under Water Stress in Zea mays L.

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P63

Drought is the major limiting factor to crop production worldwide. Root architecture can influence drought response. *Viviparous* mutant (*vp*) embryos fail to become dormant and several of the *vp* mutants are also ABA deficient. ABA level has been implicated as important in drought tolerance. Bin numbers 3.05, 4.08, and 8.05 were previously associated with QTL for primary root length and bins 1.03, 1.07, 4.04, and 5.03 with QTL for seminal root number (Gerau, unpublished). An experiment using *vp* and other mutants corresponding to these QTL regions was performed to identify genes associated with root architecture under water stress. Two experiments were conducted. In the well-watered experiment five reps were planted in a peat based growth medium. The plants were grown in a greenhouse and watered for the entire period. In the water-stressed experiment a polyacrylamide water retainer was added to the potting media to prevent desiccation of the plants. After three weeks of watering, water was withheld. Thirty-one days after planting measurements were taken on primary root length, total root mass, root branches, seminal root number, and shoot mass in both experiments. Significant differences were observed between the mutants and wild-type plants in several cases. Mutant *vp*5-DR3076, a cartenoid and ABA deficient mutant, exhibited water stress related differences in root architecture for seminal roots but not for the other parameters. The difference in seminal root number between the *vp*5-DR3076 mutant and the wild-type under well watered conditions was 3.4 while under water-stressed conditions it was 6.8. Significant differences were observed between *vp*5-DR3076 mutants and its wild type siblings in both well watered and water-stress for primary root length, total root mass, and shoot mass. The genes identified here are candidates for use in future studies of root response to water stress.

Molecular and Genetic Analysis of Mutants Causing Male Gametophytic Lethality in Arabidopsis thaliana

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P64

During pollen development, multiple archeosporial cells differentiate in the anther and divide meiotically to form four haploid microsporocytes. During the haploid phase of the male gametophytic cycle, each microsporocyte undergoes two mitotic divisions to produce a tricellular pollen grain. During this process the pollen grain precursor is progressively covered by a complex and unique cell wall that is formed after a multitude of biochemical modifications. Recently, global expression studies at the genomic level have shown that close to 10% of the genes expressed in pollen are specific to the male gametophyte; however, the functional role of the vast majority of these genes remains to be determined. We are interested in elucidating the genetic basis and molecular mechanisms that distinguish the role of essential genes during male and female gametophytic development in flowering plants. After generating a collection of transposon-based enhancer detector and gene trap lines in Arabidopsis (Sundaresan et al., 1995 Genes & Dev. 9:1797-1810), we performed a genetic screen based on the distorted segregation of the classical 3:1 mendelian ratio of a molecular marker (kanamycin resistance; KanR) carried by the mutagenic transposon element in a heterozygous individual. We analyzed 1000 enhancer detector and gene trap lines and found that 0.71% showed reduced transposon transmission through pollen. We focused our attention in four lines showing a strict and stable 1:1 KanR:KanS segregation ratio. For one of these lines reciprocal crosses showed that transposon transmission through the male gametophyte is only 0.2%, while transmission through the female gametophyte wass normal. Through routine TAIL-PCR we determined that the insertion is in a gene that encodes an alcohol dehydrogenase member of a protein family involved in cell wall biosynthesis. We did not find significant differences between mutant and wild type pollen through early developmental stages; however, close to 50% of the mutant tricellular pollen had abnormal furrows and protrusive extensions of exine. We suspect that these type of defects affect pollen viability by provoking excessive dehydration during pollen maturation and germination.

*Control of Branch Architecture in Foxtail Millet (*Setaria italica*)*

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P65

We analyzed vegetative and inflorescence architecture in the millet grasses by comparative developmental studies and by quantitative trait locus (QTL) analysis of a cross between foxtail millet (Setaria italica) and its presumed progenitor green millet (Setaria viridis). These two species differ in vegetative tillering and axillary branching, as well as in the number and number of orders of branching in the inflorescence; differences that are also seen amongst other millet grasses. We find that branching in foxtail millet is under the control of multiple QTL that are distributed non-randomly in the genome. Some QTL affect all types of branching, others only affect one aspect, such as tillering or axillary branching. Possible candidate genes controlling phenotypic effects were identified by comparative genome mapping to the maize and rice genomes, using common markers. Sequence of the rice genome that was orthologous to the QTL regions in foxtail millet was analyzed for open reading frames (ORFs), then ORFs were

translated and BLASTed to identify high similarity matches. A number of hormone biosynthesis pathway genes were identified, along with developmentally important genes in maize such as Zea floricula leafy1 (zfl1) and barren inflorescence2 (bif2), etc. Interestingly, teosinte branched1 (tb1), so important in vegetative architecture in maize, was less prominent in foxtail millet. We hypothesize that these genetic loci may be important in differentiating species in Setaria and other millet grasses, and that evolution in their temporal or spatial expression is important in creating morphological diversity.

ABA Sensing Mediates Expression of Vacuolar Invertase during Female Reproductive Development in Maize

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P66

Sucrose partitioning among maize floral tissues during the pre- and early post-pollination phases of reproductive development may be critical to the timing of female flower maturation. Sucrose cleavage by a vacuolar invertase, Ivr2A, provides hexoses essential for sink tissue growth and expansion and may be a key enzyme in the carefully- controlled, sequential expansion of maize flower tissues. The focus of this work is to examine the Ivr2A expression profile over an early developmental series under field conditions using a sensitive real time RT-PCR approach. Data show that Ivr2A expression coincides with the rapid expansion of silk and floral tissue during the pre-pollination period, yet transcript levels decline towards pollination. At this time, maximal mRNA abundance and probable hexose accumulation shifts to the ovary as flower parts senesce. This controlled pattern of expression may be significant in the timing of female floral development and consequently to silk emergence for optimal pollination. To test the possible involvement of ABA, we employed a mutant in the functional Vp1 transcription factor, classically known to cause a precociously germinating phenotype due to lack of ABA sensing. Results infer that Vp1 may play a regulatory role in the fine tuning of Ivr2A expression at these early phases of development. Sucrose cleavage by Ivr2A may be mediated through crosstalk of complex hormonal networks and Vp1 may be a central component in relaying the endogenous ABA signal in the ovary. Sampling from ABA insensitive vp1 mutants and wild type plants grown together under identical conditions allowed dissection of the interface between sugar- and ABA signaling. Analysis of vp1 mutant plants shows that the careful control and timing of Ivr2A expression among floral organs was disrupted by a lack of endogenous ABA sensing. Ovaries from these mutants express Ivr2A transcript to levels more than two-fold greater than that of wild type. These data show that an intact ABA sensing system is essential for maintaining a normal progression of Ivr2A vacuolar invertase expression within floral parts, subsequent tissue expansion, and thus optimal timing of female flowering.

Molecular and Genetic Analysis of rgh Endosperm Mutants

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P67

The maize endosperm shows much of the developmental complexity of other plant organs in addition to being an important storage reserve for the seed. An understanding of the endosperm in relation to seed specific development processes could provide a basis for developing more efficient approaches to seed improvement as well as a model for organ development in maize.

The rough endosperm (rgh) class of seed mutants disrupts normal endosperm and embryo development and is characterized by seeds with a pitted or etched surface. The development of mutagenic inbred (UniformMu) population (McCarthy et al., NSF award 007676) provide us with a unique tool for an efficient and rapid characterization of transposed tagged mutants. UniformMu is a Robertson's mutator, transposon-active population that it is introgressed into W22 color-converted inbred. Roberton's mutator elements typically have 50-200 copies/genome and can be used for efficient forward mutagenesis. So far we have identified 135 rgh mutant isolates from the UniformMu population.
We used B-A translocation stocks to locate 12 isolates in the 5L chromosome and 3 isolates in the 6L chromosome. We expect 2-3 loci per chromosome arm; allelism tests for these isolates are underway. To isolate and clone the rgh loci we are utilizing Mu-TAIL PCR to amplify Mu-flanking sequences. Candidate clones are obtain by subtracting Mu-TAIL PCR products from normal siblings of mutant Mu-TAIL PCR products. Phenotypic analysis of these isolates will be completed in parallel to the molecular and genetic analysis. This research could lead to answers many basic questions in developmental biology as well as to new tools that enhance practical uses of endosperm.

Clonal Mosaic Analysis Revealed Distinct Functions of EMPTY PERICARP2 in Maize Shoot Development

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P68

The paralogous EMPTY PERICARP2 (EMP2) and HEAT SHOCK FACTOR BINDING PROTEIN2 (HSBP2) are small maize proteins that contain a single protein-protein interaction motif: the coiled coil domain. They are accumulated differently during maize development and stress response, suggesting their functional divergence. Previous analyses with the embryo lethal emp2-R mutant revealed that EMP2 is required for regulation of heat shock protein (hsp) gene transcription in post-coleoptile stage maize embryos. Here we reported the clonal mosaic analyses of the emp2-R mutant in an effort to uncover non-redundant, post-embryonic function of EMP2. First, molecular analyses of heat stressed emp2 mutant tissues revealed no essential role in regulating HSR in young maize leaves. Second, emp2 mutant clonal sectors were associated with diverse shoot development defects including ligule/auricle displacement, altered phyllotaxy and narrow leaf. Third, equivalent phenotypes are observed in emp2-sectored plants grown under heat stress and non-stress conditions. Lastly, emp2 mutant sectors induced at different developmental stage and different location were correlated with distinct mutant phenotypes. Therefore, the molecular and morphological analyses of emp2 mutant sectors successfully uncovered additional, diverse functions of emp2 in maize vegetative shoot development, and these functions can not be compensated by the paralogous hsbp2 gene. The involvement of EMP2 in diverse maize developmental pathways also promises novel insights into these pathways by studying the biochemical function of EMP2.

Dominant Non-Reduction Mutants of Maize

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P69

Apomixis is a naturally occuring process of cloning through seeds. In the diplosporous mode of gametophytic apomixis, the female gametophyte produces an unreduced egg cell that develops into an embryo in the absence of fertilization. Thus, the embryo is an exact genetic replica of

the mother plant. Genetic experiments with Tripsacum dactyloides, an apomictic wild relative of maize endemic to Mexico, demonstrated that apomixis acts as a dominant trait that can be genetically mapped to a single chromosome arm.

One approach to uncover possible mechanisms of apomixis is to identify mutants of maize that replicate some aspects of diplospory. As mentioned above, two characteristics of apomixis are a) that it is a dominant trait that b) results in the production of unreduced egg cells. To isolate dominant, Mutator transposon-induced mutants of maize that result in the production of unreduced gametes, we took advantage of the fact that the formation of a normal seed in maize requires a 2:1 ratio of female to male genomes in the endosperm (the nutritive tissue of the seed). Thus, when Mutator-active female plants are fertilized with pollen from a tetraploid plant, normal seed will only be produced on F1 plants with dominant mutations that disrupt meiosis and yield functional unreduced female gametophytes containing a 2n egg and a 4n central cell.

Using this approach in a genetic screen of 17,000 plants, we recovered six Dominant-Non-Reduction (DNR) mutant lines. We have begun molecular and cytological analysis of two of these mutants, DNR3 and DNR4. Preliminary phenotypic analysis suggests that both DNR3 and DNR4 mutations lead to defects in chromosome condensation, segregation, and formation of the mitotic spindle during meiosis. Interestingly, similar phenotypes are observed during meiosis in apomictic Tripsacum. We will present a cytological analysis of meiosis in DNR mutants, and discuss how the phenotypes of these mutants may relate to processes that occur during apomixis.

Differential Expression of the Actin Gene mac1 in the Embryo and Endosperm During Maize Seed Development

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CINVESTAV IPN

P70

Actin cytoskeleton plays fundamental roles in different cell functions. It participates in mitosis, cytokinesis, cell shape, cytoplasmic transport of organelles and vesicles and signal transduction (Staiger C.J. 2000. Signalling to the actin cytoskeleton in plants. Annu.. Rev. Plant Physiol. Plant Mol. Biol. 51, 257-288). Cytoplasmic actin can be found in two forms: monomeric (G-actin) or in microfilaments (F-actin). These two forms are in constant interaction to achieve the distinct functions of actin. These activities depend on the different proteins that bind to actin and on the physiological state of the cell (Staiger, and Markey F., Larsson H., and U. lindberg 1982. Nucleation of actin polymerization from profilactin opposite effects of different nuclei. Biochem Biophys. Acta 704, 43-51).

In plants, the family of actin genes have been classified in two main groups according to their differential expression: reproductive genes and vegetative genes. The first ones are expressed in pollen and ovules, and the second group is expressed in vegetative tissues such as leaves, stem, and roots. Arabidopsis thaliana actin genes family is the best studied. This plant contains eight functional genes, three are reproductive, and five are vegetative (Kandasamy M.K., McKinney E.C., Meager R.B. 1999. The late pollen-specific actins in angiosperms. Plant J. 18, 681-691; and Moniz de Sa M. Drovin G. 1996 Phylogeny and substitution rates of angiosperm actin genes Mol Biol Evol. 3, 1198-1212). In maize, eight partial sequences corresponding to actin genes have been found, but the whole coding region is known only for the Mac1 gene (vegetative). However, the 5' UTR and 3' UTR regions of Mac1 were not described (4).

In this investigation, we are analyzing the differential expression of the actin gene Mac1 during maize seed formation. We are looking to Mac1 expression in embryo and endosperm at different days after pollinisation (DAP). To this end, specific primers from the 3' UTR region

from the Mac1 gene were designed and used to obtain a specific Mac1 probe for Northern blot analysis. The same tissues were analyzed by Western blot using actin antibodies.

The Globby1-1 (glo1-1) Mutation Affects Cell Proliferation and Differentiation During Early Endosperm Development

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Cereal endosperm tissues account for most of the world's caloric intake, yet the regulation of monocot seed development remains poorly understood. The maize endosperm originates with a series of free-nuclear divisions, followed by cellularization and subsequent formation of a range of functional cellular domains. Analysis of defective kernel mutants has provided some clues about how these processes occur. Of particular interest is the *globby1-1* (*glo1-1*) mutation, which induces a variety of aberrant globular kernel phenotypes by altering nuclear and cell division throughout development. Here we describe the effects of *glo1-1* on development of the basal endosperm transfer layer (BETL) and aleurone only. As a result of the mutation, cell fate acquisition and subsequent differentiation of these endosperm tissues are affected to varying degrees of severity. These findings allow us to build a clearer picture of how BETL and aleurone cell fate and differentiation occur in the maize endosperm.

Ragged Seedling2 Leaves Fail to Expand Despite Retention of Adaxial/Abaxial Polarity

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Current models state that lateral expansion of leaves results from juxtaposition of adaxial (Ad) and abaxial (Ab) domains and that reduction of either domain results in reduced expansion. ragged seedling2 (rgd2) phenotypes, however, do not readily support these models. Radial rgd2 leaves retain Ad/Ab polarity and narrow leaves do not exhibit an overall reduction of either Ad or Ab features, although Ad and Ab epidermal tissues may exhibit features of their opposite sides and vascular bundles are rotated out of alignment with respect to the mediolateral plane of the leaf. NARROWSHEATH expression and faulty KNOX protein accumulation in rgd2 shoot apical meristems suggest that leaf development is affected early in these plants by failure of cells to adopt the proper state of determinacy. Coincident with this failure is a reduction in the expression of a maize YABBY homologue. YABBY genes are normally expressed in expanding regions of leaf primordia in maize and Arabidopsis and may be involved in the progression to developmental determinacy. Taken together, these data support the conclusion that rgd2 leaves poorly communicate positional information, possibly as a result of establishing improper states of determinacy in leaf primordia.

INVUNCHE, An ISWI-like Chromatin Remodeling Factor Essential for Megagametogenesis and Early Seed Development in Arabidopsis thaliana

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CINVESTAV-IRAPUATO

P73

Recent studies identified some of the mechanisms responsible for altering chromatin structure and allowing interactions between regulatory proteins and genomic DNA in plants. We are interested in elucidating the function of the Imitation of Switch (ISWI) class of ATPases during megagametophyte formation and seed development in Arabidopsis thaliana. Members of the ISWI class of ATPases regulate chromosomal assembly and transcriptional regulation during at least two flowering pathways. We determined the pattern of expression of 3 members of the ISWI family by reverse transcription PCR (RT-PCR). All 3 genes are expressed in most vegetative and reproductive tissues (flower buds, mature flowers, unpollinated and pollinated gynoecia, and siliques). To determine if ISWI genes have a specific role during female reproductive development, we modified the pFGC5941 dsRNA vector (kindly provided by Jorgensen's group, U. of Arizona) and replaced the CaMV35S promoter by pFM1, a 844 bp regulatory sequence that drives expression only in the megagametophyte and the young seed. The original and modified vectors were used to clone palyndromic coding sequences and silence each of the ISWI genes by RNA interference (RNAi). Transformants using the CaMV35S promoter for a particular ISWI-like gene (INVUCHE;INV, an infant male from Chilo's mitology, Chile), showed reduced vegetative growth and aborted seed formation with variable degrees of penetrance. Aborting seeds contain defective embryos arrested at the mid-globular stage. These embryos have aberrant patterns of cell division in the embryo-proper, poor differentiation of the protoderm, and additional cells in the suspensor. The endosperm does not form following fertilization. In-situ hybridization shows that INV is expressed during ovule and megagametophyte development, with high levels of transcript localization in the nuclei of haploid cells. After fertilization INV is expressed in both the embryo and the endosperm. Transformants using the pFM1 specific promoter showed megagametophytes consistently arrested at either the 2- or 4-nucleated stage. Our results indicate that INV is essential for the second and third haploid nuclear divisions in the megagametophyte and regulates both early embryo patterning and the initiation endosperm development in Arabidopsis.

The Maize Duplicate Gene Narrow Sheath2 Encodes a Conserved Homeobox Gene Function in a Lateral Domain of Shoot Apical Meristems

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The narrow sheath (ns) phenotype of maize is a duplicate factor trait conferred by complimentary mutations at the unlinked loci ns1 and ns2. Recessive mutations at each locus together confer the phenotypic deletion of a lateral compartment in maize lateral organ; no phenotype is observed in plants homozygous for a single ns mutation. Previous morphological studies, KNOX (KNotted1-like homeobOX) immunolocalizations, and clonal analyses of narrow sheath and non-mutant plants suggest that maize leaves are comprised of at least two distinct lateral compartments, which define the central and narrow sheath domains of maize lateral organs. These studies suggested a model in which NS function is required to recruit organ

founder cells in a lateral compartment of maize meristems. Cloned by homology to its duplicate gene narrow sheath 1, the narrow sheath 2 gene encodes a predicted homeodomain protein with homology to the PRESSED FLOWER1 protein of Arabidopsis thaliana that is required for the development of lateral sepals and sepal margins. Analyses of NS protein accumulation verify that the ns-R mutations are null alleles. Quantitative and in situ expression assays reveal that ns1 and ns2 transcripts accumulate in a developmental-specific manner in tissues enriched for vegetative and reproductive shoot meristems, and lateral organ primordia. Moreover, previously undiscovered leaf and stamen phenotypes in the PRESSED FLOWER1 mutant support a model whereby the morphology of eudicot leaves and monocot grass leaves has evolved via the differential elaboration of upper versus lower leaf zones. The data are discussed in terms of a model implicating an evolutionarily conserved NS/PRS function during recruitment of organ founder cells from a lateral domain of plant meristems.

Interactions Between XCL1 and KNOX Genes: A Hormonal Connection

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P75

An important question in plant developmental biology is how division and differentiation are balanced in shoot apical meristems (SAMs) in order to maintain meristematic cells and to allow for the production of leaf primordia from the flanks of the meristem. KNOX genes play an important role in the maintenance of meristem identity. In simple-leafed model species such as maize and Arabidopsis, class 1 KNOX genes are downregulated in the P0 and remain turned off throughout leaf development. Dominant, neomorphic mutations in maize KNOX genes such as KNOTTED1 (KN1), lead to the production of knots of tissue over veins and ectopic ligules which require periclinal cell divisions in epidermal cells (Gelinas et al., 1969). In addition, the severity of kn1 loss-of-function phenotypes is worsened when the mutation is introgressed into inbred backgrounds with shorter meristems (Vollbrecht et al., 2000), indicating that KN1 plays a role in maintaining meristem size. Extra cell layers1 (Xcl1) is a semi-dominant, hypermorphic mutation that causes the overproduction of a normal gene product and affects cell division and differentiation patterns in developing leaves and kernels. Oblique, periclinal divisions in the protoderm give rise to multiple epidermal layers in leaves. In addition, Xcl1 meristems are 60% shorter than normal sibling meristems, and recruit more cells into P0 (Kessler et al., 2002). The short meristems and increased recruitment of cells into P0 seen in Xcl1 mutants may reflect compromised KNOX activity. Double mutant analysis between Xcl1 and dominant KNOX mutants indicate that Xcl1 is a suppressor of the Kn1 phenotype, possibly through modulation of GA biosynthesis. Xcl1 displays a synergistic phenotype when present with Gnarly1 and Rough sheath1. These double mutants phenocopy polar auxin transport inhibitor-treated seedlings, indicating that Xcl1 may affect auxin transport in meristems. Thus, XCL1 provides a link between KNOX and hormonal signaling pathways.

Mutations in the MADS Box Genes ZMM8 and ZMM14 Are Associated with an Indeterminate Floral Apex Phenotype

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Maize inflorescence architecture is controlled by the activity of inflorescence meristems during development. Both the identity and determinacy of inflorescence meristems are affected in the

indeterminate floral apex1 (ifa1) mutant and in plants homozygous for Mu insertions into the ZMM8 gene. These mutants have a loss of determinacy in the Floral Meristem (FM), Spikelet Meristem (SM) and Spikelet Pair Meristem (SPM) of the tassel, displaying extra floral organs, extra florets and an extra apex between rudimentary silks. MADS box genes are involved in floral development in a wide range of plants, affecting both organ identity and meristem determinacy. Many lines of evidence indicate that the ifa1 phenotype is caused by a mutation of the MADS box gene ZMM14, an idea supported by the almost identical phenotype seen in ZMM8-Mu insertion line. Plants carrying both mutations show a more severe lack of determinacy, suggesting that the loss of both of these closely related genes reduces meristem determinacy more than a loss of either gene alone.

The Role of the Maize Gene, Thick Tassel Dwarf1, in Inflorescence Architecture

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In an effort to examine floral organ formation in maize, we analyzed the thick tassel dwarf1 (td1) mutant in detail. Mutants show an increase in spikelet density in the ear and tassel, an increase in single spikelets in the ear and tassel, and extra floral organs (especially stamens). Spikelet meristems do not appear to be as severely affected. Mutant plants are slightly shorter depending on inbred background, suggested that td1 also affects the vegetative meristem. Mapping data placed the maize orthologue of the Arabidopsis gene, clavata1, in the same bin (5.03) as the td1 mutant. To determine if td1 is a clavata1 gene, the reference allele was sequenced and alleles that were obtained by directed transposon tagging were analyzed for the presence of Mu insertions. The original td1-r allele contains a small deletion and 5 independent Mu insertion alleles were identified. Cosegregation analysis further confirmed that td1 is the maize clavata1 gene. Northern analysis revealed that td1 is expressed in the vegetative shoot, ear and tassel.

A Reverse Genetic Approach to Find New Members of the ERF Family of Transcription Factors Involved in Maize Inflorescence Development

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P78

The ERF family is a large family of plant transcription regulators. They are involved in key developmental steps and in stress response. BRANCHED SILKLESS1 (BD1), an ERF transcription factor, plays a fundamental role in maize spikelet development controlling meristem identity. A reverse genetic approach was taken in order to clone new ERF factors involved in development of maize inflorescence architecture. A maize cDNA library from a baby ear was screened using the BD1 DNA binding domain as a probe. 15 clones coding for putative ERF transcription factors were isolated. Northern blot and RT-PCR analysis narrowed down the pool of clones to three new ones that showed specific expression in ear and tassel. Interestingly, all three genes show a high similarity among each other at the DNA and protein level. In addition, all of them have an EAR repression domain at the C-terminus. To investigate their possible action in the BD1 pathway, their expression was tested in different bd1 mutants. The expression of all three genes is down-regulated in the bd1 mutant alleles. The level of

expression matches the strength of the bd1 allele: almost no expression in a bd1 null allele and partial expression in a weak allele. Moreover, promoter analysis of these three ERFs shows the presence of at least one BD1 binding site. Binding of BD1 to these promoter sequences was confirmed by a gel shift assay. A better understanding of the role of these transcription factors will come from analysis of the Mu insertion alleles that have been found for at least two of the three genes.

The Egg Apparatus-Specific Peptide ZMEA1 From Maize is Required to Guide the Pollen Tube Towards the Female Gametophyte

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P79

With the aim to identify genes specifically upregulated in the unfertilized egg cell of maize, a differential plaque screening and sequencing of some 1,000 ESTs was conducted with a cDNA library of maize egg cells. Among the isolated clones, ZmEA1 (Zea mays egg apparatus) represents a large cluster with 32 members (3.3% of all egg cell ESTs). ZmEA1 does not show any significant homology to known genes, proteins or ESTs in public data bases and is specifically expressed in the egg apparatus (egg cell and synergids) of maize, as shown by RT-PCR analysis and in situ hybridization. ZmEA1 maps on chromosome 7L and is not co-localized with known phenotypic markers.

In order to study the subcellular localization of the predicted ZmEA1 peptide (94 aa containing a predicted transmembrane domain), a construct consisting of the maize ubiquitin promoter driving the expression of a ZmEA1-GFP fusion protein was transformed by particle bombardment at onion epidermal cells. ZmEA1-GFP localization was observed in vesicles accumulating at the cell surface, in transvacuolar strands and endoplasmic reticulum surrounding the nucleus. A GUS construct containing some 1.5 kbp upstream of the transcription start point (full length promoter) was transformed into maize, to study ZmEA1 promoter specificity. In addition, three different GUS promoter deletion constructs as well as one barnase construct were generated and transformed into rice. The full length promoter was also used to drive ZmEA1-GFP fusion protein expression in transgenic maize plants. GFP fluorescence was observed exclusively in the micropilar region of the maize embryo sac where the pollen tube arrives.

RNAi and antisense constructs were generated to silence ZmEA1 in maize, with the aim to study the function of the gene. In vitro pollination tests were conducted to estimate the fertilization efficiency: pollen of GUS-expressing lines were used to fertilize transgenic ZmEA1-RNAi maize cobs and wild type maize cobs, as a control. We will demonstrate that the fertilization efficiency was less than 50% for the RNAi transgenic cobs compared to controls and show that ZmEA1 is required to guide the pollen tube towards the egg apparatus of maize.

Corn Smut Induced Maize Genes

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P80

Ustilago maydis the causal agent of corn smut disease induces the formation of enlarged galls (tumors) on any above ground portion of its host, maize. We have identified fungal mutants that are unable to cause gall formation but are able to cause symptoms that typically lead up to gall formation, for example chlorosis and anthocyanin production. Employing a comparative

approach between wild-type infections (galls) and mutant infections (no galls) we are identifying maize genes up- or downregulated during gall formation. The method we have primarily used involves suppression subtractive hybridization PCR (SSHP). Because of the induction of highly modified structures (galls) in the plant, it is likely that some of the genes identified in this screening will represent genes positionally or temporally misexpressed in specific tissues or developmental stages. To date we have identified several genes upregulated during gall formation and we are in the process of more fully characterizing the temporal expression of a few select genes. One gene that will be presented as an example encodes a putative ETA subunit of the CCT chaperonin. This chaperonin subunit has been implicated in mammals to be critical for the function of cyclin E which in turn is essential for progression from G1 to S phases of the cell cycle. This suggests that corn smut infection is inducing changes in plant gene expression commensurate with typical phenotypic outcomes.

Cloning Extended auricle1, an Essential Component in Maize Leaf Development

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P81

We have characterized a novel mutant eta, extended auricle, which is involved in proximodistal patterning in the maize leaf. The most noticeable phenotype is an extension of auricle tissue into blade and a distal displacement of the blade/sheath boundary. While the eta phenotype is very similar to the suite of dominant Knotted1-like homeobox (knox) mutants, it is a recessive mutation and does not ectopically express knox genes. However, eta acts as an enhancer in combination with proximodistal patterning mutants like Kn1, Lg3, Gn1, Rs1and rs2. eta also has a synergistic interaction with lg1 and a dosage effect with lg2. In addition, eta appears to interact with mop1, a gene involved in paramutation of the paramutable color genes B', Pl', and R' as well as the epigenetic regulation of the Mutator family of transposons. SEM analysis reveals Eta+ functions early in leaf development to properly establish the blade/sheath boundary. Eta is a unique gene as it appears to interact with several different genetic pathways in maize leaf development, but the genetic sequence of Eta is still unknown. We describe two approaches to cloning Eta: one approach is map-based cloning with SSR molecular markers and restriction fragment length polymorphisms (RFLPs). The second approach is co-segregation analysis of putatively Mutator-tagged eta alleles.

Gene Expression Profiles from Isolated Egg Cells and Pro-Embryos of Wheat

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P82

The fertilized egg cell represents a totipotent cell which develops into a functional multicellular organism. In most animal species studied, maternally stored mRNAs in the egg cell are involved in the establishment of embryo axes, diversification of cell types and morphological changes during early embryogenesis. Following fertilization and throughout embryogenesis, transcript profiles are dynamically changing and transition from maternal to embryonic control takes place. Compared to animals, little is known about the transcript composition of the female gametes (egg cell and central cell) from seed plants or about transcriptional changes occurring during very early embryogenesis. Using microdissection, we are isolating egg cells and central cells from unpollinated wheat ovules, as well as defined stages of zygotes and early embryos after hand-pollination. cDNA populations were generated out of a few reproductive cells and

subsequently used to study gene expression profiles by restriction fragment differential display PCR (RFDD-PCR) as well as by bioinformatical analysis of some thousand ESTs. The expression pattern and function of selected candidate genes was further investigated by RT-PCR and knock-outs using the RNAi approach. A detailed report will be given about genes specifically expressed in egg cells and proembryos, representing potential key genes involved e.g. in cell identity, signalling, fertilization or the establishment of the egg cell and/or embryo polarity.

Analysis of the Expression Pattern and Regulation and Probably Function of AGL12, a MADS-Box Gene Involved in Development of Arabidopsis thaliana

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P83

MADS-box genes are coded by a big family of transcriptional factors that in plants play important roles in development. Their study has been made mainly in flower development, but very few is known in vegetative development. In Arabidopsis thaliana, there are at least 11 MADS-box genes that are expressed preferentially in roots. AGL12 is one of them, and by in situ analysis the transcripts of these gene are expressed in the external cells of the columella and in lateral root cap (LRC) cells. In the proliferative region there is a punctuated expression in the atrichoblast cells and in the differentiated zone the expression is detected in the vascular bundle. To analize in a specific way the expression pattern of AGL12 in roots, we made a constructs that has 2.8 kb of the promoter region fused to GUS/GFP, and we found that AGL12 is expressed in the floem. But by RT-PCR experiments we found that AGL12 is also expressed in other tissues, like flowers and fruits. To define the possible function of the gene in the plant development we have a mutant line got it by transposon insertion, and over expression lines (2xp35S::cDNA AGL12). The analysis of the mutants phenotype has shown, that the roots growth less than the wild type line. When we made analysis at cellular level, we found that the differences in growth are because there is a different cell production rate which is the result of different number of cells in the meristem.of the mutant line. In the aerial part of the plant, the agl12 mutant present a delay in the flowering time of about 18 days, however, there are not changes in the floral whorls, all of them are present and in the correct position.

Obtainment and Molecular Characterization of Transgenic Cuban Maize Highly Resistant to Spodoptera frugiperda Smith *Attack*

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CIGB

P84

In the present study, we describe the obtainment of self-pesticide transgenic maize plants of the Cuban commercial variety FR-28 designed to resist Spodoptera frugiperda Smith (fall armyworm) attack, the most important pest of maize in Cuba. The insecticidal properties have been conferred by the introduction of synthetic versions of Bacillus thuringiensis genes coding for either Cry1Ca or the interdomain hybrid Cry1Ab-1Ca toxins (formerly H04), which have been found as highly specific for fall armyworm insects. Calluses containing organogenic and

embryogenic-like structures (O'Connor-Sanchez et al, 2002) were used as the plant material for genetic transformation via Agrobacterium tumefaciens. Hundreds of clones have been regenerated from ammonium glufosinate resistant calli and characterized through molecular analysis (PCR) and bioassay using neonate fall armyworm larvae. Several clones were able to cause death to fall armyworm larvae after 72 hours of exposition with minor damage to foliar tissue. Other caused a delay in the normal growth. Seeds from the most resistant clones were germinated in a second generation under controlled conditions following restricted biosafety rules to select the most prominent clones displaying high level of resistance to fall armyworm attack for future field trials.

Beta-Expansins in Maize Pollen: Role of Zea m 1 in Development and Fertilization

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P85

The Group-1 grass pollen allergens from maize pollen, called Zea m1, make up a sub class of beta-expansins, which are cell wall loosening proteins that loosen plant cell walls in characteristic ways. Group-1 pollen allergens are specifically expressed in grass pollen and are proposed to loosen the cell walls of the silks to aid in the penetration and growth of the pollen tube through the silks. Previous work shows there are two divergent classes of Zea m1, A and B, each class containing two genes. In our current efforts, we are screening BAC libraries and have identified 9 BAC clones that contain at least 14 Zea m1 genes; 10 in class A, and 4 in class B. Thus, the maize genome contains more copies of Zea m1 genes than previously appreciated.

We have started work to identify in situ localization of Zea m1 transcripts and protein using short radiolabeled probes and immunohistostaining on male flowers at various developmental stages. Results of this work will be presented at the meeting.

To assess the function of Zea m 1, maize plants were transformed with an RNAi construct for RNA silencing, driven by a pollen specific promoter. Many lines in the first generation were lethal. Those who survived have the following unusual traits: low pollen production, shriveled anthers, continued silk growth post pollination and low seed set. These phenotypes are more extensive than that found previously for a maize line carrying a Mu insertion in one of the Zea m 1 genes. These results indicate an additional role of Zea m1 in pollen development.

OCL Genes Are Involved in the Determination of Kernel Size in Maize

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The OCL1 to OCL5 genes (Outer Cell Layer) encoding putative transcription factors are expressed in the L1 cell layer of the embryo and several other tissues of maize (Zea mays). OCL1 was isolated by a differential display between microspores and androgenic embryos, genes OCL2 to OCL5 by cross-hybridisation with OCL1. The protein sequence revealed the presence of a homeo domain (DNA binding) at the N-terminus, followed successively by a leucine zipper (protein/protein interaction), a START domain (steroid binding) and a conserved domain with unknown function at the C-terminus.

The expression of the five genes is more or less restricted to the L1 cell layer of the embryo, the endosperm, the apical meristem, leaf primordial and young flowers. Although the expression territories of the 5 genes showed big overlaps, each gene had a distinct spatial expression pattern.
Eight insertions of the transposon Mutator in four OCL genes were obtained by screening of the Pioneer TUSC collection of 40 000 mutagenised plants. Although some of the insertions were located in exons, no strong phenotype was observed in any of the homozygous mutants.
In contrast transgenic plants carrying a fusion of the repressor domain of the Drosophila engrailed gene with the DNA binding and dimerisation domains of OCL1 showed a temporal reduction of kernel size.
In a effort to identify potential direct or indirect target genes of OCL1, a microarray-based transcriptome analysis has been initiated. Fluorescently labelled cDNA were prepared from OCL1-engrailed and wildtype ovules and hybridized to a maize unigene microaray.

Dynamics of Aleurone Cell Formation: The Surface Rule

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P87

Aleurone cells, sometimes referred to as the epidermis of the endosperm, cover the inner body of starchy endosperm cells in maize. On the outside, the aleurone cell layer is juxtaposed by maternal tissues, viz the nucellar epidermis cell layer. Of the three known genes implicated in aleurone cell fate specification and maintenance, Cr4 (Becraft et al. Science 273: 1406) and Dek1 (Lid et al PNAS 99: 5460) encodes a TNFR-like receptor and a calpain like cystein proteinase that both are predicted to be located in the plasma membrane. The third gene, Sal1 (Shen et al. PNAS 100: 6552) encodes a homologue of the human Chmp1 protein, a vacuolar processing enzyme implicated in endosome trafficking mediated regulation of the density of cell surface receptors. In this poster we report on observations of endosperm with developmental defects from a microscopy screen of hand sections of non-plump grains from the Pioneer TUSC collection. In mildly defective grains, the endosperm often contain crevasses in the surfaces penetrating into the starchy endosperm cell mass. In all cases, these crevasses were coated with aleurone cells. In more severe cases od developmenta defective endosperms, the body of endosperm cells only partially filled the maternal endosperm cavity. In all cases, the endosperm cell mass was organized into a surface layer of aleurone cells and an inner mass of starchy endosperm cells. From these observations we infer that the endosperm posses an intrinsic ability to *self organize* into a surface layer of aleurone cells and an inner mass of starchy endosperm cells. The exact role of the cr4, dek1 and sal1 genes in this process is currently unknown.

Assessing the Functional Redundancy in the Maize C-Class Control of Carpel and Stamen Identity

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P88

In Arabidopsis and Antirrhinum, the ABC model describes the patterning of a flower, in which discrete classes of transcription factors act alone or in combination to specify the identity of the organs in each whorl: sepals, petals, stamens, and carpels. The C-class is necessary for stamen and carpel identity as well as for conferring determinacy to the floral meristem. Arabidopsis has a single C-class gene AGAMOUS(AG), and ag mutants show loss of floral

meristem determinacy and homeotic conversion of stamens to petals, and in the place of carpels a new flower develops (reiterating the sequence sepal, petal, petal, sepal, petal, petal …). A search for AG homologues in maize has yielded three genes: ZeaAGamous1 (ZAG1), Zea Mays Mads2a (ZMM2a) and ZMM2b. ZMM2a/b are a recent duplication, while the ZAG1/ZMM2 duplication is more ancient. Previously, we used reverse genetics to isolate a zag1-mum1 knock out showing a loss of meristem determinacy but little or no organ identity defects. This partial C-class mutant phenotype in zag1-mum1 suggests that ZMM2 is not completely functionally redundant with ZAG1. Furthermore, the non-identical expression patterns we observe suggest sub-functionalization of the gene activities. In order to test the hypothesis that their protein activities have diverged in function, we have used the maize AG homologs to rescue the Arabidopsis ag mutant. Our results indicate that in fact their activities are sub-functionalized: ZAG1 is better able to rescue carpel identity, while ZMM2 is better able to rescue stamen identity. This suggests sub-functionalization has occurred at the level of protein activity in addition to the gene expression. Experiments are under way to identify the region of the protein that defines this difference. We have also recently identified a putative ZMM2a loss of function allele, and work is under way to characterize its effect on maize flower development.

Isolation of the New Root Mutant rum1 Affected in Lateral and Seminal Root Initiation

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P89

Two thousand Mu-tagged F2-families were visually screened for aberrant root phenotypes 10 days after germination in paper rolls. A new monogenic recessive mutant designated rum1 (rootless with undetectable meristems 1) was identified in B73 background. The mutant rum1 is affected in the initiation of lateral roots and seminal roots. Histological analyses of cross sections of embryos 30 days after pollination showed that the mutant does not develop seminal root primordia. This distinghuishes the mutant rum1 from the previously described lateral root initiation mutant lrt1 (Hochholdinger and Feix, Plant J. 16: 247-255, 1998) which develops normal seminal roots. Like lrt1 the mutant rum1 is affected in the initiation of lateral roots of the primary root, but not in shoot-borne root development and lateral roots that are emerging from these roots.

Lateral roots are formed through dedifferentiation of already differentiated pericycle-cells via the formation of root primordia and meristems. Gene expression profiles of pericycle-cells of wild-type and rum1 primary roots were studied via laser capture microdissection (LCM) and subsequent cDNA microarray-experiments. Statistical analyses identified genes that are preferentially expressed in wild-type or rum1 pericycle expression. Further analysis of these differentially expressed genes is in progress.

Fate and Consequence of the ZAG1/ZMM2 Gene Duplication Across the Grasses

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P90

Gene duplications are a prevalent feature of eukaryotic genomes. Understanding the evolution of gene duplications is important to our understanding of genetic redundancy and the evolution of new gene function. We present data on two developmentally important duplicate genes, ZAG1 and ZMM2, that appear to be key determinants of stamens and carpel specification in

grasses. ZAG1 and ZMM2 are maize orthologues of C-function organ identity genes as defined in the ABC model of flower development. In maize, ZAG1/ZMM2 appear to have partitioned C-function activity through the evolution of sub-functionalized coding and non-coding cis-regulatory regions. Using phylogenetic methods, we have identified the origin and distribution of the ZAG1/ZMM2 duplication in grasses, and we have characterized the expression patterns of ZAG1 and ZMM2 in specific grass taxa, and in select non-grass species that diverged prior to the ZAG1 and ZMM2 duplication. Our data indicates that the duplication occurred just prior to the diversification of the grasses, with closely related non-grass lineages having only a single ZAG1/ZMM2 gene. Both genes appear to have been preserved in all of the grass lineages, suggesting selection for retention of both gene activities early in the history of this duplication event. Our preliminary gene expression data, in situ hybridizations and RT-PCR analyses, suggest that taxa closely related to the grasses that have only a single copy of ZAG1/ZMM2 show gene expression in both stamens and carpels. Among those species where both genes are present, when expression of ZAG1 and ZMM2 has been examined, both genes are expressed differentially. ZMM2 is expressed in both male and female organs throughout development, whereas ZAG1 is expressed in both organs early in development but in later development is restricted to the carpel. We are continuing to study the subfunctionalization of ZAG1 and ZMM2 using a combination of gene expression methods such as in situ hybridizations and RT-PCR and transgenic techniques using Arabidopsis as a heterologous system to uncover differences in C-function activity.

Genetic Analysis of Root Responses to Phosphate Starvation in Arabidopsis thaliana (L.) Heynh

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P91

In response to phosphate (Pi) starvation, plants had evolved sophisticated adaptative mechanisms at morphological, physiological, biochemical and molecular level to acquire Pi from rhizosphere. One of most remarkable morphological responses is the change in the root system architecture (RSA), which includes changes both in root growth rate and root hairs, as well as lateral roots induction and distribution. Additionally, several putative components of a Pi starvation-inducible rescue system has been described, including high-affinity Pi transporters, secreted phosphatases, and conserved short open reading frames coding for RNAs of unknown function. Many of these adaptations are triggered as specific and direct responses to Pi deprivation, thus giving a fundamental role to mechanisms by which cells sense and response to changes in Pi availability. Nevertheless, still is not known how this system senses neither environmental cues nor transduction pathways that integrate diverse responses in Pi-starved plants.

Present work were conducted to identify genes involved in RSA modification of Arabidopsis thaliana as response to Pi starvation, trying to isolate mutants that exhibit alterations to common adaptative responses, which in turn may lead to unravel components of this process.

Based upon a morphological screening set up in our group. Arabidopsis seedlings were grown in under sterile conditions for 12 days in modified MS with high phosphate (5mM) KH2PO4 supply. We selected mutant lines (both insertional and EMS) displaying phenotypic modifications or changes in RSA associated to Pi-starved plants in optimal supply or insensitive to starved conditions, which were further analysed over main root architectural features. As a complement to this strategy we analysed expression patterns of selected Pi-starvation-inducible

(PSI) genes (ACP5, AtPT2, AtIPS1 and PAP1)by Northern blot and RT-PCR in our lines. Genes were selected by their specific up-regulation as well by their different temporal induction over time to integrate responses to drastic morphological changes in RSA.
In lines displaying Pi-starved RSA in sufficient Pi supply, we determined that specific genes are up-regulated even at this optimal condition, which may be an evidence of the involvement of our mutants in the normal process of adaptative responses. In contrast to this behaviour, lines displaying Pi-insensitive RSA in Pi-starved conditions, has shown down-regulation of PSI genes in the stimulating condition (e.g. Pi starved) or up-regulation in non-inductive condition (e.g. Pi sufficient), thus reinforcing the hypothesis that alterations of normal RSA changes in response to Pi starvation are part of a coordinated strategy of the plant to cope with detrimental conditions, and linking deregulation of morphological to molecular responses at temporal level. The results support the notion of a putative plant pho regulon system, and diversity of genetic elements involved from sensing to signalling and response to cues provided both from internal and external signals, in which our mutants are involved. Current efforts in our lab are focused on cloning insertional mutants and positional mapping of EMS lines to finally elucidate the function of selected lines in the process, and to have more clues to understand the complex response of plants to Pi starvation.

Epigenetics Posters

Chromatin Genes: Discovery, Mutagenesis, and Function

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P92

In plants, there are many examples in of genes whose expression is regulated epigenetically by chromatin packaging. However, the mechanisms controlling chromatin-based expression are not completely understood. Our goal is to define and analyze the genes in maize that regulate gene expression via chromatin-mediated processes.
We have taken several steps to reach this goal. Using the sequences of known chromatin-based regulatory genes in yeast, mammals and Arabidopsis, we have searched maize sequences to identify genes with sequence similarity to other chromatin factors. We have mapped the genes genetically to describe copy number and chromosomal location, and we have used RNA blots to determine the level of gene expression in different tissues. Finally, we have used an RNA interference (RNAi) strategy for obtaining mutants in these genes, and we are conducting assays of the mutants to determine how they affect chromatin-level expression. Genes, map positions, RNA data and mutant status are available at http://www.chromdb.org.
Our results indicate that maize contains a large number of genes with motifs like those found in chromatin genes in other organisms. Most of the maize genes are duplicated in the genome and many are members of multi-gene families. A number of the genes show tissue-specific patterns of expression. Some, but not all, of the RNAi-induced mutants have reduced levels of expression of the targeted genes, and a subset of these have discernible effects on development and / or expression of reporter genes known to be subject to chromatin-mediated regulation.

Role of Chromatin Remodelling Factors During Female Gametogenesis in Arabidopsis thaliana

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P93

The formation of the female gametophyte requires the establishment and maintenance of a precise pattern of cell fate. To control cell fate and gene expression in a flexible way, plant cells have developped robust epigenetic mechanisms that play pivotal functions in plant development. In Arabidopsis thaliana, several genes encoding chromatin remodelling factors have been shown to be involved in reproductive development; however, their potential role during female gametogenesis remains unknown. We are investigating the gametophytic role of members of the SWI/SNF2 gene family: PIKLE/GYMNOS, SPLAYED, MOM1 and MOT1, as well as the Arabidopsis homologues of the human ySNF2 and ATRX genes, which belong to distinct clades of the gene family tree (available at www.chromdb.org). We are also including in our study FAS2, a gene encoding a subunit of the CAF-1 complex involved in maintenance of meristem cell fate by regulating nucleosome structure. We have taken advantage of the RNA interference (RNAi) strategy to inactivate each of these genes using a promoter specifically acting in the female gametophyte. pFM1 is a 844 bp regulatory sequence that was obtained from an enhancer detector line showing reporter gene expression in the functional megaspore stage on, until full differentiation of the female gametophyte. We modified the dsRNA vectors generated by the group of R. Jorgensen (U. of Arizona) by replacing the CaMV35S promoter with pFM1, and transformed wild-type individuals with palyndromic RNAi constructions corresponding to each of our candidate genes. After plant transformation with each of four constructs (PKL-pFM1:RNAi, FAS2-pFM1:RNAi, MOT1-pFM1:RNAi and MOM1-pFM1:RNAi), between 30 and 83 T1 plants resistant to the herbicide BASTA were analyzed. Among the first populations of T1 plants we found between 6 and 14% showing a female sterile phenotype. In all cases ovule abortion appeared to be related to the developmental arrest of the female gametophyte at the functional megaspore stage and a subsequent degenerescence of the gametophytic cells. These results suggests that female gametophyte development is likely to be highly sensitive to modifications of chromatin organisation regulatory mechanisms.

Imprinting of the Maize Endosperm Specific Gene fie1 Is Mediated by Demethylation of Maternal Complements

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P94

The maize fie1 gene, a homolog of the arabidopsis FIE (fertilization independent endosperm), is specifically expressed in the endosperm and not in other tissues. Only the maternally transmitted fie1 allele is active in the endosperm, suggesting imprinting control over fie1 gene expression. To understand the role of DNA methylation in silencing of the paternal fie1 allele, DNA methylation assays were performed with methylation sensitive enzymes HpaII/MspI and bi-sulfite sequencing on DNA samples isolated from the endosperm, embryo and leaves. Both methods detected cytosine methylation at CG and CNG sites in the fie1 promoter and in exons 1 - 8. However, downstream exons 10 - 12 were not methylated indicating a localized pattern of DNA methylation within the upstream region of the fie1 gene. This pattern of methylation was found in all tissues tested. Using SNPs (single nucleotide polymorphism) between B73 and Mo17 parental lines in fie1 exon1 we demonstrated that the maternally transmitted fie1 alleles

were unmethylated in the endosperm, but the paternally transmitted allele was methylated. Both parental alleles were methylated in the embryo and leaf DNAs that correlates with silencing of the fie1 gene in these tissues. Data suggest that the methylated state is the default for the fie1 gene in all tissues tested, except in the endosperm where transcriptional activation of the maternal fie1 is achieved through demethylation. The paternal allele remains methylated and transcriptionally inactive during endosperm development.

Epigenetic Stability at the Maize pl1 Locus

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P95

The Pl-Rhoades (Pl-Rh) allele of the maize purple plant1 (pl1) locus participates in paramutation, a form of heritable epigenetic gene silencing. As pl1 encodes a myb-like transcription factor that controls anthocyanin biosynthesis, pl1 gene activity can be easily monitored with a visual assay. Pl-Rh can exist in a spectrum of epigenetic regulatory states conferring very little to robust amounts of pigment. Strongly expressed states of Pl-Rh are invariably altered, in a heritable manner, to a weak expression state (referred to as Pl'-mahogany or Pl') when carried in heterozygous combination with another Pl-Rh allele in a Pl' state. The ability of one Pl-Rh allele to facilitate trans-silencing (referred to as paramutagenic activity) is inversely correlated with pl1 RNA levels (Hollick et al. 1995). Fully expressed Pl-Rh expression states (referred to as Pl) are largely stable and non-paramutagenic. Pl-Rh is unique in that all other pl1 alleles tested appear not to participate in paramutation. Sequence comparisons suggest that Pl-Rh has unique structural arrangements required for epigenetic modulations. Stability of the Pl' state requires the presence of at least two Pl-Rh alleles; Pl' is unstable in Pl\' / - hemizygotes and can revert to a higher activity state (Hollick and Chandler, 1998). While no other pl1 alleles aside from Pl-Rh have been found to acquire paramutagenic activity, genetic analyses suggest two classes of pl1 alleles exist - (1) alleles that efficiently maintain a heritable Pl' state in a pl1 / Pl' heterozygote, and (2) alleles that allow reversion of Pl' to a higher activity state in a manner similar to a pl1 deficiency. Loss of function derivatives of Pl-Rh have been generated to functionally identify cis-elements required for acquisition of paramutagenic activity and the maintenance of Pl\' stability. Genetic analyses of these derivative alleles coupled with comparative studies of diverse pl1 alleles suggest that cis-elements required for the acquisition of paramutagenicity and maintenance of Pl' stability may be separate.

Analysis of Tissue Culture-Induced White Cob Mutants Define Mechanisms of Epigenetic Change Induced by Stress

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P96

Plant cells in tissue culture undergo a dynamic genomic reprogramming and regenerant-derived progeny exhibit a high frequency of heritable mutations. This reprogramming process can be manifested by genetic and/or epigenetic changes. We have been characterizing white cob mutants originating from tissue culture. A tissue-culture induced c2 allele is due to a small palindromic insertion. An update on the analysis of this insertion will be presented. A detailed analysis of tissue-culture induced P-ww alleles will be presented. The P-ww derivatives of P-wr are interesting because suppression has occurred across all copies of this tandemly repeated locus indicating a concerted mechanism to silencing. In addition, the P-wr allele appears

sensitive to silencing in culture based on the frequency of P-ww alleles relative to other genes in this pathway. A detailed methylation analysis describing extensive stretches of CG and CNG methylation in these mutants relative to the control will be presented. These studies provide insight into mechanisms of epigenetic silencing in tissue culture, and support the idea that stable epigenetic change can underly stress-induced phenotypic variation.

RNAi-Mediated Silencing of Maize Chromatin Genes and Their Effects on Maize Transformation and Genomic Methylation

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P97

Chromatin remodeling plays a crucial role in gene regulation and expression. A more complete understanding of the role of chromatin effects on transgene integration and expression is needed to facilitate plant transformation experiments and to understand mechanisms of lateral DNA transfer. The goal of this study was to characterize the effects of dsRNA-mediated silencing of chromatin genes on transformation efficiency. Inverted-repeat (IR) constructs targeting CHR106, HON102, CHR110, and GTC101 were preliminarily determined to confer increased or reduced transformation efficiency during the process of a routine transformation experiment. These constructs were used in replicated transformation experiments to confirm their effect on the frequency of transgenic events produced. IR constructs containing the maize chromatin gene CHR106 and HON102 showed a 4X and 2.3X increase, respectively, in transformation over the empty vector pMCG161. Conversely, a 1/3X reduced transformation efficiency was obtained with the IR construct for the gene GTC101. Three constructs targeting CHR106 and its homolog CHR101 (homologs of Arabidopsis ddm1) were used in our experiments, one specific to each gene, and the other silencing both. Transgenics from the construct both the CHR101 and CHR106 show reduced methylation of repetitive sequences. Constructs which target only CHR101 or CHR106 do not show reduced methylation of repetitive sequences, yet CHR106 gives an enhanced transformation phenotype. Our interpretation at this point is that CHR101 and CHR106 are functionally redundant with respect to repetitive sequence methylation in callus, but reduction of CHR106 provides a unique benefit during the transformation process.

Rmr6 Functions in Paramutation and Developmental Epigenetics

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P98

The rmr6 (required to maintain repression6) locus encodes one of several trans-acting factors required to maintain paramutation-induced silencing at the purple plant1 (pl1) locus. The pl1 gene encodes an R2R3 Myb-type transcriptional activator that regulates the expression of genes required for anthocyanin biosynthesis. The Pl-Rhoades (Pl-Rh) allele can adopt various epigenetic states of expression that are directly reflected by visible levels of pigmentation. Strong expression states (referred to as Pl) undergo transcriptional repression when coupled in a heterozygote with weak expression states (referred to as Pl'), an example of paramutation (Hollick et al. 1995; Hollick et al. 2000). Pl-Rh alleles that segregate from a Pl/Pl' heterozygote remain repressed and direct silencing of naive Pl states in subsequent progeny (Hollick et al. 1995). Molecular and genetic analyses indicate that RMR6 is necessary for the maintenance of Pl' repression. Pl'/Pl' plants with an rmr6 mutant genotype have high levels of anthocyanin pigmentation. Run-on transcription experiments demonstrate that these plants have increased

pl1 transcription rates and pl1 transcript levels, relative to non-mutant siblings. Also, approximately 24% of Pl' alleles transmitted from rmr6 mutant genotypes lose the capacity to repress Pl states.
In addition to its role in maintaining epigenetic repression of Pl', RMR6 ensures proper plant development. Phenotypic analyses implicate RMR6 involvement in plant growth and apical internode elongation, establishment of abaxial/adaxial polarity, floral organ identity, and lateral meristem repression. RMR6 also ensures proper tissue-specific expression patterns of specific regulatory factors. Double mutant analysis suggests that RMR6 restricts sk1 (silkless1) expression from the apical inflorescence. Similarly, RMR6 appears to restrict pl1 expression from aleurone tissues, a site where the paralogous colorless1 (c1) factor is expressed.
The action of RMR6 in pl1 paramutation and its important role in developmental progression supports the hypothesis that the epigenetic regulation of paramutation is related to normal mechanisms of developmental gene regulation (Brink 1964).

Ufo1 Induces Global Gene Up Regulation in Maize Pericarp

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P99

DNA methylation is implicated in gene expression modifications through a complex network of proteins that participate in chromatin remodeling. To understand regulation of DNA methylation, we have used alleles of the maize p1 gene that differ in their methylation and correlative expression patterns in pericarp and cob glumes. P1 is an R2R3 type of MYB factor that regulates transcription of flavonoid structural genes which catalyze the biosynthesis of red phlobaphene pigments. We have recently characterized a dominant modifier called Ufo1 (Unstable factor for orange1) which reduces the methylation of specific p1 alleles (Chopra et al., 2003, Genetics 163:1135-1146). Ufo1 is an unlinked factor (maps on 10S) from p1 (maps on 1S) and its presence with P1-wr allele causes abnormalities such as stunted growth, leaf rolling, leaf spotting, and a downward curvature of the stem. In the current study, we performed microarray analysis to measure Ufo1- induced global changes in gene expression in the presence of the P1-wr (white pericarp and red cob) allele. The microarray data revealed that 65% of the differentially-expressed genes were up-regulated, while 35% were down regulated ($p<0.05$). Our results confirm the up-regulation of certain phenypropanoid genes that were previously shown to be required for phlobaphene synthesis. Interestingly, our data analysis also shows transcriptional modification in genes that possibly play a role in epigenetic regulation of gene expression. In addition, we found that several cytoskeletal genes are strongly up-regulated in Ufo1 pericarp. The cytoskeleton is important in many physiological responses including cell growth, elongation, and gravity sensing, and as mRNA scaffold for protein translation. A model will be presented that explains how Ufo1 may function to modify gene expression.

Genetic and Molecular Characterization of Interaction of Different Alleles of p1 with a Dominant Epigenetic Modifier Ufo1

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P100

In maize, the pericarp color1 (p1) gene encodes a Myb-homologous transcription factor that regulates the synthesis of red phlobaphene pigments in kernel pericarp (outer layer of ovary wall) and cob glumes (palea and lemma). Multiple alleles of p1 are named based on the pigmentation of these tissues e.g. P1-rr plants have red pericarp and red cob, where as P1-wr

plants possess white pericarp and red cob glumes. The differential expression patterns of p1 alleles have been attributed to differences in gene copy number and/or methylation status. P1-wr is composed of six, tandemly repeated copies which are hypermethylated than the single copy allele P1-rr (Chopra et al., 1998, MGG 260:372), whereas P1-pr (patterned pericarp, red cob) differs from P1-rr only for its hypermethylated state (Das and Messing, 1994, Genetics, 136: 1121). In order to understand the epigenetic regulatory mechanisms and their role in differential expression of p1 alleles, we characterized a dominant modifier Unstable factor for orange1 (Ufo1; Chopra et al., 2003, Genetics 163: 1135). We observed that presence of Ufo1 causes decrease in DNA methylation of P1-wr leading to pigmentation of pericarp and other plant parts which are normally colorless in wt P1-wr plants. Sibling progeny plants showed a range of DNA methylation changes that correlated with the degree of pigmentation. To further understand the role of Ufo1, we have now characterized its interaction with other p1 alleles which differ in copy number or extent of methylation. Analysis of these interactions will be presented. Theses studies will allow us to understand if Ufo1 elicits its function through gene copy number or DNA methylation at different p1 alleles.

CpNpG Methylation Reduction in Plants Homozygous for the Chromomethylase Mutant Allele zmet2:m1 Is Sequence Dependent

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P101

Four classes of DNA methyltransferase in plants control maintenance and de novo cytosine methylation. Class I DNA methyltransferases act during DNA replication to maintain symmetrical CpG methylation across cell divisions. Class II DNA methyltransferases, chromomethylases, contain a chromodomain and are responsible for the majority of symmetrical CpNpG and some asymmetrical CpNpN DNA methylation. The class III DNA methyltransferases encode enzymes for de novo methylation and are responsible for the majority of asymmetrical CpNpN and some CpNpG DNA methylation. Class IV DNA methyltransferases are related to the mammalian DNMT2 gene and their function is not completely characterized. Recent evidence suggests that both class II and class III DNA methyltransferases are required for proper CpNpG methylation patterns at certain loci and that these enzymes may have some overlapping function. The goal of this research was to characterize patterns of CpNpG methylation in plants containing the chromomethylase mutant allele zmet2:m1. We have demonstrated that this mutant has a 30-50% decrease in CpNpG methylation and alters CpNpG methylation at highly repetitive sequences. In this study, we further define methylated sites affected by this mutant using probes including Class I and Class II transposable elements, low copy genic sequences, and additional classes of repetitive elements. Our results demonstrate sequence specificity to methylation changes in these mutant lines. This analysis further defines the role of specific methyltransferases in determining methylation patterns in maize.

The Maize Polycomb Group Gene, Mez1, Shows Imprinted Expression Throughout Endosperm Development

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P102

The Arabidopsis gene Medea is an important regulator of endosperm development. The expression of Medea displays an imprinted pattern of gene expression with only the maternal

allele expressed in the endosperm. To date, no ortholog of Medea has been detected within any monocot species. A detailed analysis of the maize genome identified three Enhancer of zeste-like genes; Mez1, Mez2 and Mez3. Mez1 is most closely related to the Arabidopsis Clf while Mez2 and Mez3 are most closely related to Eza1. There is no Medea-like gene detected in the maize genome. We performed allele-specific expression analysis of Mez1 and Mez2 in developing maize embryos and endosperms to determine whether either of these genes is imprinted. Mez2 was biallelicly expressed in all tissues tested. Mez1 displayed biallelic expression in the developing embryo. However, only the maternal allele of Mez1 was detected in the endosperm. Maternal monoallelic expression of Mez1 was documented for all alleles tested. The imprinting of Mez1 suggests that Mez1 may be providing Medea-like functions in maize. The promoter sequence and structure of Mez1 is compared to that of other promoters for imprinted genes. This documents an example of the convergent evolution of imprinting within a closely related gene family in plants. The finding that at least one Enhancer of zeste-like gene is imprinted in both maize and Arabidopsis provides support for the requirement of imprinting of this gene product.

Paramutation: Long-Range Epigenetic Interactions in Maize

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P103

We investigate the molecular basis of paramutation. Paramutation is a mitotically and meiotically heritable change in the expression of one allele caused by a trans interaction with another allele. Our paramutation model system, the b1 locus in maize, involves trans communication between alleles, long-range regulatory interactions in cis and the establishment of heritable epigenetic states. These processes are important elements in gene control in many higher eukaryotes and are associated with DNA methylation and specific changes in chromatin structure.

Recombination experiments showed that a sequence region about 100 kbp upstream of the b1 coding region is essential for b1 paramutation (Stam et al., 2002, Genes & Dev. 16:1906; Stam et al., 2002, Genetics 162:197). This region is at the same time required for the activity of a b1 enhancer, which activates the b1 promoter 100 kb downstream, indicating the involvement of long range communication between sequences far upstream and the promoter proximal region. This upstream region contains seven 853 bp tandem repeats that are required for both paramutation and high expression. The recombination experiments also indicated that sequences between 8.5 and 49 kb upstream of the b1 coding region contain regulatory sequences required for the basal level of b1 expression. We hypothesize that enhancer sequences ~100 kb upstream interact in cis with the b1 promoter proximal region, either directly or via the basal regulatory sequences.

Results show that b1 paramutation involves specific changes in DNA methylation and chromatin structure (nuclease sensitivity). DNA methylation differences correlate with paramutation. Interestingly, there are C nucleotides that are only methylated in the low expressing state, as well as a C nucleotide that is only methylated in the high expressing state. Based on the behavior of these different type of sites in mutant backgrounds affecting paramutation, we speculate that the differential methylated regions have different functions. Some sites might be involved in enhancing expression, while others are involved in paramutation. Paramutation correlates with changes in nuclease sensitivity. Which chromatin structure changes are correlated with paramutation is currently studied in more detail.

In parallel with the studies in maize, the paramutation system is being transferred to Arabidopsis to facilitate the identification of components that are involved in this process. As a first step we have generated a number of Arabidopsis transgenic lines in which the tandem repeats were fused to a reporter gene to ask whether the repeats contain enhancer activity. None of the sequences tested gave enhancement of expression relative to control fragments. DNA methylation analyses of the transgenic repeats suggest that the transgenic repeats are in the suppressed state, which might explain the lack of enhanced expression. In order to revert the repressed state into an active state, the transgenic sequences are being manipulated in mutant backgrounds relieving transcriptional gene silencing. The enhancer sequences might also be located outside of the repeats. To examine this possibility, a transgenic construct containing 110 kb of sequence upstream of the b1 coding region is being generated.

Centromeric RNAs are a Component of Maize Centromeric Chromatin

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P104

Although highly divergent in sequence and size, long tracts of tandem repeat arrays form the centromeres in maize and many other complex eukaryotes. In maize, these consist primarily of a ~156 nucleiotide non-coding satellite repeat, CentC, and a class of centromere-specific retrotransposons, CRM. These repeats have been shown to interact with the centromeric histone variant CENH3, a marker of a functional centromere. Here we use the technique of chromatin immunoprecipitation (ChIP) to demonstrate a biochemical association between maize centromeric repeat RNAs and CENH3. Additionally, CentC transcripts greater than 15 kb are observed in preparations of total RNA. Endogenous promoters in CRM elements may be driving read-through transcription of the centromere proper. The resulting RNAs are strongly associated with centromeric chromatin and may, by analogy to animals, be involved in structure and/or recruitment of the functional kinetechore.

Allelic Effects of Maize Chromomethylase Mutants on DNA Methylation

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P105

Chromomethylases are plant specific DNA methyltransferases that control CNG methylation. We have previously reported on the effects of the zmet2:m1 allele on methylation patterns in maize. In this study, we have characterized additional Mutator alleles of Zmet2 (a.k.a. DMT102) as well as an allele of Zmet5 (a.k.a. DMT105), a homolog of Zmet2. These alleles were identified in Pioneer Hi-Bred's TUSC population. The 5' UTR region was a hotspot for Mu insertion with eleven of twelve additional insertions occurring within a 300 base pair region. A novel insertion allele into the first exon produced a methylation phenotype identical to zmet2:m1 corroborating the phenotypic effect of the zmet2:m1 allele. Analysis of zmet2/zmet5 double and single mutants is underway to characterize potentially overlapping effects of the two genes.

Genomic Structure & Synteny Posters

Cytogenetic Mapping of Maize with Sorghum BAC FISH Probes

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P106

The maize genome is divided among 10 chromosomes, and gene locations can be charted as genetic, physical, or cytological maps. The production of triply-integrated maps for maize would provide useful knowledge and reagents for understanding and manipulating the structure of the maize genome. We present a new project to produce a cytogenetic map of the entire maize genome (NSF-DBI-0321639) using sorghum BACs as FISH probes on pachytene spreads. The approach was shown to work for three test loci on chromosome 9 (Koumbaris & Bass, 2003, Plant J. 35(5):647-659). We are scaling up this approach to develop a cytogenetic map of all ten maize chromosomes, with public data release being coordinated through the MaizeGDB. We have already begun to examine the value of these novel materials and methods by using them in conjunction with two different maize genome projects - one involving interphase FISH detection of rearranged chromosomes (W.F. Sheridan) and another involving indirect localization of genetically mapped active Ac elements (T.P. Brutnell).
The main goal of this project is to FISH map 500 genetic loci by 2006.
The core specific aims are:
(1) To FISH map the core marker loci (~ 10/chromosome),
(2) To FISH map poorly centromere-linked loci (~6-8/chromosome),
(3) To FISH map UMC98 backbone loci (at ~ 2-5 true-cM intervals),
(4) To FISH map other loci or regions of value to the maize genetics community.
Early efforts will focus on developing a detailed map of maize chromosome 9. In addition, an outreach project, the Maize-10-Maze project, will use the new cytogenetic map to guide the production of a field replicate of the maize genome, implemented by local science education leaders. The status and progress the cytogenetic map of maize project will be presented and potential applications of the technology will be discussed.

High-Throughput Anchoring Of Bac-Based Physical Maps Of Maize to Sorghum, Rice And Sugarcane

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P107

With many plant species entering the beginning phases of genome sequencing and BAC-based physical mapping, both comparative biology and cost-efficiency can be fostered by the use of tools that are portable to diverse taxa. We present a high-throughput approach to anchor BAC-based physical maps of multiple species, both to genetic maps and to one another. Synthetic 'overgo' probes were simultaneously anchored to BAC libraries of *Sorghum bicolor, Sorghum propinquum, Zea mays, Oryza sativa, Musa acuminata* and *Saccharum* (hybrid). Overgo probes were designed based on the most conserved 40 bp segment of a target sequence, as determined by cross-species hits to GENBANK (excluding repetitive DNA) and SUCEST (Private Sugarcane EST database). Overgo probes were hybridized in multiplex arrays with up to 24 probes per pool and the results deconvoluted. A total of 31 high-density BAC filters from seven different libraries totaling over 500,000 BACs were tested on at least a portion of the probes. Hybridization and BAC fingerprint data are being used to construct physical maps using the program FPC. To date, over 7,000 probes have been tested on *Sorghum* while 2672 probes have been tested on *Maize.* About 1/3 of all of the probes are based on a 2,509 locus sorghum genetic map, and the remainder comprising BAC end sequences or specific genes of interest. The resulting physical maps can be used to transfer information from the rice sequence to other grasses. This will be demonstrated by comparison of the sorghum genetic map to the rice and

Maize physical maps. We will also review the feasibility of developing overgo sets that work on many diverse angiosperms, based on early results from cotton, *Brassica* and *Sorghum*.

High-Resolution Genetic Mapping of Chromosome 1 in Maize after Ten Generations of Recurrent Intermating in the IBM Population

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P108

The Intermated B73 x Mo17 (IBM) syn10 population was developed and evaluated as a high-resolution mapping population. Additional generations of random mating increase the probability of observing recombination between linked loci. The two populations compared in this study were IBM syn4, with four generations of random intermating after the F2, and IBM syn10, with ten generations of random mating intermating after the F2. Segregation data for 94 IBM syn4 lines and for 94 IBM syn10 lines were collected at 75 SSR loci along chromosome 1. The best loci order was estimated using MapMaker and recombination fractions were calculated and adjusted to a single meiosis basis. The additional six generations of random mating increased this chromosome 1 genetic map length from 462.7cM in IBM syn4 to 865.1cM in syn10. The 1.53-fold increase in recombination fraction observed in the syn10 population resulted in a better ability to order the loci. Within the chromosome 1 map, the additional recombination in syn10 also caused some intervals between previously linked loci in syn4 to approach a recombination fraction of 0.5 in syn10. To adjust for the increased recombination fraction, a framework map with a higher marker density than used in this study is needed. Because of increased recombination between linked loci, the IBM syn10 population is a suitable resource for projects requiring higher-resolution mapping, such as positional cloning, QTL mapping and marker-assisted selection.

Chromosomal Relationships Defined by Repetitive Sequence Profiles

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P109

Maize chromosomal repetitive element profiles show that each chromosome shares a similar composition with another chromosome. Oat-maize addition lines, each with an individual maize chromosome added to a complete oat genome, allow the abundance of maize-specific sequences to be quantified for each maize chromosome. Microarray technology and DNA from the oat-maize addition lines has allowed the relative composition of selected repetitive sequences to be evaluated on a chromosome-by-chromosome basis. Forty-two probes designed to 17 maize repetitive elements were created and used to survey the maize portion of the oat-maize addition lines. Three oat probes and dilutions of Avena sativa and Avena ventricosa genomic DNAs were used for internal controls and normalization. Comparisons with previous measurements of the 180 bp knob specific sequence (Ananiev et al., Genetics 149: 2025-2037) correspond with this approach. Both t-tests and F-tests show significant differences in the abundance of the various repetitive sequences between different maize chromosomes. Chromosomes paired by similar repetitive sequence compositions agree with pairings proposed in previous studies of the macro-duplications within maize defined by genic data. Agreement of chromosome pairings created by genic or repetitive sequence information may shed light on the evolutionary history of the maize genome. This material is based upon work supported by the National Science Foundation under Grant No. 011134.

IBM Neighbors -- Mutual Enhancement of Genetic and Physical Maps

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P110

Assembly of the maize physical map relies heavily on a well-ordered genetic map. While the best map for this purpose is the high resolution IBM map (1,2), it does not include all possible anchor sites for the physical map. We have developed a tool that extracts map data from MaizeGDB (http://www.maizegdb.org/), and computes an approximate IBM coordinate for loci not placed on that map. Users may weight the computation in favor of maps with best statistical support; the algorithm automatically weights in favor ofon-frame order, assigned by mapping software used to compute the original maps. The algorithm relies on maps that share loci, either with the IBM, or some other map that shares loci with IBM. We have restricted the initial content to maps with (a) better than bin resolution and (b) markers that support physical map anchoring. The June 2003 version contained 5700 loci, representing nearly 7000 RFLP, SNP, IDP (InDel; insertion deletion polymorphism) and SSR probes. Using the Jan 2, 2004 physical map (http://genome.arizona.edu/fpc/maize/), the current version includes an additional 15,000 sites, representing cDNA-derived overgos placed onto anchored contigs. The approximate orders, derived from strictly genetic order data, have been refined by physical map order. IBM neighbors may be explored at MaizeGDB, Gramene cMap (http://www.gramene.org/), TIGR (http://www.tigr.org/), and the Maize Mapping Project iMap (http://www.maizemap.org/), where software may be freely acquired. This project was supported by NSF grant #DBI 9872655.

(1) Lee et al 2002 Plant Mol Biol 48:453-461
(2) Sharopova et al 2002 Plant Mol Biol:463-481

Comparative Analysis of Ramosa1 Gene Function in Maize, Sorghum and Rice

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P111

We are investigating developmental, molecular and evolutionary bases of inflorescence architecture diversity, by identifying inflorescence architecture genes in maize and then analyzing these genes within a comparative framework in the grasses. In the maize *ramosa1-ramosa2* pathway, mutation of either *ra1* or *ra2* causes replacement of short branches (spikelet pairs) by long branches on main axes of the tassel and ear. Most of the extra long branches in mutants bear multiple, single spikelets ('spikelet multimers'); thus, *ra1* and *ra2* function as spikelet pair genes. We previously showed that *ra1*, which encodes an EPF zinc finger protein and is likely a transcriptional repressor, is genetically downstream of *ra2* and that *ra2* regulates accumulation levels of *ra1* RNA. Across an allelic series of *ra1* and *ra2* mutants, degree of branching correlates inversely with *ra1* RNA levels. Furthermore, *ra1* nucleotide diversity is greatly reduced in maize relative to *Tripsacum* and teosinte, a signature of a domestication gene. These findings suggest that differences in *ra1* function may associate with natural variations in grass inflorescence architecture. In support of this hypothesis, two cases of high branch number correlate with delayed or reduced *ra1* expression relative to the reference RNA expression profile of *ra1-B73*. First, a large QTL for branch number maps to *ra1* in the Illinois High Oil (IHO) line. *ra1-IHO* fails to complement the *ra1-R* mutation, IHO tassels develop as do mutant *ra1-R* tassels and *ra1-IHO* expression is delayed during tassel development. There are just a few SNPs in five kb around *ra1-IHO* and present evidence leads us to speculate that this heterochronic allele may be due to epigenetic effects. Second, in the highly branched

inflorescence of *Sorghum bicolor* diverged from maize ~ 20 mya, expression of the *ra1* ortholog (*ra1-Sb*) is delayed and reduced relative to *ra1-B73*. In extending our comparative analysis to rice, whose inflorescence branches are multi-spikelet, database searches with *ra1* did not return an unambiguous ortholog among rice EPF family members. We therefore selected and sequenced a sorghum BAC and compared the genomic sequence with the orthologous genomic segments of maize (a BAC contig) and rice (genomic sequence). A tandem duplication of *ra1-SB* and promoter rearrangement suggests a basis for its delayed RNA expression. Microcolinearity between sorghum and rice is conserved with only one exception: *ra1* is absent from the rice segment. Gene order in the maize BAC contig is also consistently microcolinear with that in sorghum. Thus, in this genomic segment and since the last common ancestor of the panicoids and rice (50 - 70 mya), *ra1* was either gained in the lineage leading to the panicoids or lost in the lineage leading to rice. We favor the latter hypothesis because there is no unequivocal *ra1* ortholog elsewhere in rice and absence of *ra1* function in rice is consistent with its long branch architecture. We also present a progress report on efforts to clone *ra2* by transposon tagging in maize.

Comparative Analysis of a One-Megabase Sequence Spanning the Maize Rf1 Fertility Restorer with the Rice Genome

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P112

In T-cytoplasm maize, cytoplasmic male sterility (CMS) is attributed to the presence of the unique mitochondrial gene, T-urf13. Full suppression of T-urf13-mediated CMS is directed by the combined action of dominant alleles of the nuclear (fertility restorer) genes, rf1 and rf2. As a first step in the positional cloning of the rf1 gene, one-megabase of maize chromosome 3 spanning the rf1 locus was sequenced and annotated. Three B73 BAC libraries were used to create a physical map of 439 clones anchored to the rf1 locus. A minimum-tiling path of 12 sequenced BACs comprise 79% transposable elements as well as other repetitive sequences, including centromeric-specific repeats. One-hundred fourteen unique gene families from the megabase maize sequence were compared to the TIGR rice pseudomolecules. One-hundred sixty one maize sequences that correspond to rice genes were found dispersed throughout the genome, suggesting that large-scale synteny has dissipated since species divergence. In contrast, mico-colinearity is still intact, shown by groups of multiple genes that have retained relative distribution and orientation in both species. Additionally, several single copy maize genes from the rf1 region are found in multiple positions on the rice genome, restricted to either single chromosomes or particular areas within chromosomes. This sequence contig facilitates a candidate gene approach towards identification of the rf1 family while also providing a basis for comparison of functionally related regions in cereals.

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Genomics Posters

Integrative Genomic Analyisis in Mexican Forage Maize

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Maize (Zea mays L.), the most economically important grain and forage crop in Mexico, produces highly nutritious grain, forage and forms an integral link in diversified sustainable agricultural system. To be profitably grown, forage maize must produce high levels of biomass, both stem and leaf, good nutritional value and animal performance, and increased metabolites content to be conserved for long time periods. Several environmental and genetic factors determine yield and persistence under intensive system of production. The long-term goals of our research program are to understand the genetic basis of biomass and quality value in forage maize, annual and model plant species, and specifically to clarify the role of genomic, proteomic and metabolomic into the biomass and nutritive value. Our experimental genetic material consists of three double haploid (DH) populations derived from six representative Mexican forage maize inbred lines contrasting in flowering cycle, yield, height and quality value; 30 hybrids derived in a diallel design with reciprocal crosses also from the six inbred lines; and 60 open pollinated, hybrids and inbred populations representing the gene pool of tropical forage maize. The construction of genetic maps in the three DH haploid populations derived from 150 SSR polymorphic pair primers, and the phenotyping of spring establishment, summer forage growth, and tissue physiological and biochemical characteristics will allow us to estimate quantitative genetic parameters and identify quantitative trait loci (QTL) for various components of morphological and metabolite forage maize traits. Synthesis of QTL positions for these traits with map locations and expression profiling will enable us to develop a genetic framework for the forage maize genome landscape, including identification and characterization of potential candidate genomic regions (www.chapingo.mx/ForageGenomic/). The identification of the marker sequences (SSR) derived from EST markers associated to metabolic traits are located, and will lead us to identify candidate genes. The 30 hybrids are being marker screened to search heterotic patterns of forage quality and metabolite profile. We also present an approach using factor analysis as a tool for dimensionality reduction and metabolite modeling to estimate the molecular genetic variation of 60 populations. Results have direct applicability to forage maize breeding programs via marker-assisted selection, candidate gene and allele approaches and further genetic engineering.

Dynamic Nature of the Integration of Plastid Sequences into the Mitochondrial Genome

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It has been known for two decades that plastid DNA (ctDNA) is incorporated into the maize mitochondrial genome. Sequencing of four maize, two teosinte and one sorghum mitochondrial DNAs (mtDNA), and comparisons among these genomes have revealed that plastid-to-mitochondrion transfer is both more extensive and more active than previously appreciated. For instance, the previously described 12 kb segment of maize ctDNA present in mtDNA represents only the largest part of a 23 kb region from the plastid inverted repeat that now exists as multiple

fragments in the maize mitochondrial genome. The presence or absence of any specific portion of this region is highly variable among taxa. In another example, two sequences that are widely separated in the plastid genome are catenated in the mitochondrial genome. The catenation of the two regions appears to have been the result of recombination between rpl23 sequences that are present in each. Consistency in the organization and rearrangement of this transferred DNA in the grass mitochondrial genomes implies a single transfer event that occurred BEFORE the divergence of these grasses. Phylogenetic analyses, on the other hand, indicate that these transferred sequences are most closely related to those of the plastid of the same taxon rather than to transferred sequences in other taxa, and imply multiple transfers AFTER the divergence of the taxa. These two factors together suggest an early initial transfer and integration event, followed by copy correction after the divergence of individual grass taxa. More generally, we propose that the integration of exogenous DNA into the plant mitochondrial genome is a rare event, but that recombination of newly-acquired exogenous DNA with pre-existing homologous sequences is relatively common.

This work has been supported by National Science Foundation grant DBI-0110168.

The Effectiveness of GeneThresher™ Methylation Filtering Technology in Sorghum and Its Comparison to Maize

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Orion Genomics

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The maize gene space is currently being sequenced by High Cot and GeneThresher methylation filtering technologies, which target the low copy and hypomethylated regions of the genome, respectively. There is approximately a 1x coverage of the hypomethylated gene space by the methylation filtering component of the project. We have also obtained similar coverage of the Sorghum bicolor gene space by methylation filtering. The maize and sorghum data sets were compared to a comprehensive set of rice coding sequences and similar coverage was obtained. This suggests that they contain comparable gene representations, which is consistent with the similar gene space coverage. We have completed a comprehensive analysis of the sorghum GeneThresher data which should be directly applicable to the maize set. The sorghum data shows an unbiased coverage of all gene regions (5'regions, exons, introns, and 3' regions) and tags approximately 90% of sorghum genes. Additionally, the maize GeneThresher set annotates sorghum genes missed by the sorghum set. This highlights the benefits of comparative genomics in the grasses.

Identification of Early Expressed Genes and Genes Expressed Differently in B73 and Mo17 after UV Radiation

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Understanding the mechanisms plants use for acclimation and adaption to ultraviolet radiation stress is key in predicting plant responses to our changing environment. Study of UV perception has been complicated by uncertainty about whether a particular response reflects damage/inhibition or photoreceptor activation. Although diverse UV responses have been measured (growth, shape changes, photosynthetic parameters, etc.) it has been difficult to pin down the underlying mechanisms. We used cDNA arrays (unigene 1 arrays from the maize gene discovery project) to analyze UV-induced alterations in gene expression, as a step in identifying the underlying mechanisms for UV responses. We determined which genes are

significantly affected by UV radiation and how expression is altered with increasing UV exposure. By comparison of leaf responses in two inbreds used in gene mapping studies we have identified candidates for genetic analysis of allele contributions to UV responses.

Construction of Libraries and Analysis of ESTs From a Phosphorus Efficient-Zea mays Line Grown Under Low-Phosphorus Stress

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In response to low-phosphorus, Zea mays develops biochemical and morphological processes to optimize and increase the internal concentration of this element. In order to identify genes involved in phosphate uptake and utilization efficiency and to define the expression profiles of such responses, we have constructed 3 subtracted cDNA libraries from roots and shoots grown hidroponically with optimal (driver) or limiting (tester) phosphorus. Tissue was isolated from roots at day 1, 3 and 6 after the beginning of the stress (ABS), and from shoots at day 3 ABS too. Such times match to the onset of many biochemical and morphological changes that were determined through expression kinetics of a marker of the response and adventitious-root development kinetics under limiting phosphorus. Additionally, a complete cDNA library from root tissue of 3 days ABS was constructed. At that time, responses at the biochemical level are evident. 259, 67, 308, 163 and 3837 cDNAs were randomly selected from each library and sequenced from the 5¬¥ end, resulting in average lengths of 570 for the subtracted and 3837 bp for the complete libraries, respectively. The ESTs were assembled using the program CAP3, resulting in 56, 63, 30, 88 and 2718 unigene clusters. When were compared with the non redundant database, the BLAST search analysis (e value <1e-06, bitscore >80 identity >80%) showed that several unigene clusters have significant homology to maize, rice and Arabidopsis genes, whereas a relevant percentage of clusters from each library showed no significant homology even comparing with the EST-others database. The functional characterization based on the gene consortium assignments is being performed.

Integration of Genetic and Physical Data in 2,585 Contigs

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High-resolution genetic maps and deep physical maps of maize, anchored inter se, are ready for targeted research and for tiling and sequencing. The 2,300 Mb genome assembles readily, even though highly duplicated and rife with recently diverged, nested retrotransposons and other repetitive sequences. From HindIII, EcoRI, and MboI BAC libraries of B73, 290,000 agarose BAC fingerprints (15X) were assembled initially into contigs using FPC. Phase I manual editing for the June 2003 build drew together 3,500 contigs assembled at 10e-12 with the aid of an end-joining utility and over 15,400 markers, including 9,300 unigenes addressed to the BACs with 40bp overgos. Physical addressing of genetically mapped markers by PCR on 48x48x48, 6-dimensional BAC pools proved to be an efficient and trustworthy aid. Targeted SNP mapping is on EST unigenes that are associated with unanchored contigs. These data and tools have

largely anchored the contigs, some exceeding 8 Mb, to the genetic map. Contig order and orientation devolves from over 2000 markers mapped genetically at high resolution, which give virtually perfect correlation with physical order. A 'neighbors' map, derived by intercalation of loci from other informative populations, combines over 5,800 mapped loci in approximate order, and assists editing. Phase II editing for the January 2004 build drew together 2,585 contigs with 18,649 markers, representing 2050 Mb of the genome, 56% anchored to the genetic map. Software tools to systematize, to validate, to link, to pipe, and to integrate large, diverse, high-throughput datasets are available. See www.maizemap.org and genome.Arizona.edu/fpc/maize/ for views and downloads. Side-by-side dynamic views of the physical and genetic map from a database environment are in the iMap presentation at www.maizemap.org/iMapDB/iMap.html. Supported by NSF #9872655.

EST Sequencing Efforts at CINVESTAV-Irapuato

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CINVESTAV

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As part of the functional genomics program of our institute, we recently established a laboratory with an intermediate capacity for DNA sequencing, microarray printing, reading and bioinformatic analysis. In the case of maize we are interested in sequencing ESTs of plants subjected to abiotic stress such as drought and low phosphate availability and the early stages of female gametogenesis. For generating cDNA libraries of plants subjected to abiotic stress we selected lines that have been identified as tolerant to drought or having a high efficiency of P assimilation. Conventional and subtracted cDNA libraries were produced from root and leaf tissue of plants at different stages of the stress treatment. To determine ESTs derived from the early stages of female gametogenesis, ovules at the stage of megaspore formation and during cellularization of the embryonic sac were isolated and total cDNA libraries were produced. Our goal is to determine the s√©quence of at least 20 000 genes in order to determine which of those genes are differentially expressed under relevant stress conditions prevalent in Mexico, as well those involved in reproductive development and/or grain formation.

For this purpose we have generated 8 substractive and 5 standard cDNA banks. The standard cDNA were cloned in a vector that allows expression in yeast and have an average insert length above 600 bp. All banks have been ordered using a Chip Writer Colony Arraying-Colony Picking System (Virtek) in 96 well plates. Plasmid DNA is prepared using QIAprep96 systems (QIAGEN), yielding 15 ,Äì20¬*µ*g DNA per clone, and sequencing is carried out using a 96 capillary system (ABI 3700) with the Kit Big Dye Terminator v3.1 Cycle sequencing from ABI.

A total of 25,248 sequencing reactions have been carried out generating 25,138 raw sequences (approx. 99% efficiency). From the raw sequences 20,482, around 81% are high quality. Our most recent clustering (29 Dec 2003) indicated that we have 10,468 clusters obtained from 20,153 sequences showing 48 % redundancy. All information generated has been deposited in our still not publically available data bank at our computer system 'Mazorka'. In the near future we expect to sequence another 25,000 additional clones from 5 new standard cDNA banks and design microarrays with the unigene collection generated.

Polyphyletic Origins of Cultivated Rice from Pre-Differentiated Ancestors

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In contrast to the monophyletic domestication of maize (Zea mays), cultivated rice (Oryza sativa) appears to have polyphyletic origins. If the indica and japonica sub-species of rice correspond to independent domestication events, the divergence between the sub-species may predate domestication. Thus, it is of interest to know whether the sub-species represent independent domestications from a single progenitor population, or whether each sub-species was derived from a unique population. In this study, we use 60 nuclear SSRs, chloroplast gene sequences, nuclear gene sequences, and transposable element-based anchor markers to assess the relationships between the major groups of O. sativa and the species commonly thought to be their wild progenitors, Oryza rufipogon and Oryza nivara. Our results reveal population structure within O. rufipogon and suggest groups more closely related to each of the rice sub-species. As seen in previous studies, our analysis shows five readily distinguishable groups within O. sativa: aus, indica, aromatic, tropical japonica, and temperate japonica. Further, the rooting of phylogenetic trees with the wild species confirms the previously reported close relationship of the aus rice varieties with the indicas, and the relationship of the aromatic varieties to the japonicas. This assessment of population structure within cultivated and wild rices is a prerequisite for using these populations in association mapping studies. Additionally, the ability to detect differences between groups of wild Oryza species that are not clearly discernable phenotypically is valuable for allele mining and germplasm conservation efforts.

Host Effects of a Susceptible and a Resistant Maize Line on the Replication and Movement of the Sugarcane Mosaic Virus

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The severity of infection caused by sugarcane mosaic virus (SCMV) in maize plants varies depending on the maize genotype and the viral strain. This might reflect the many different interactions between proteins from the host (associated with resistance or susceptibility) and those from the virus (avirulence or virulence), which determine the level of resistance in the plants. One example is the restriction of replication or movement, either local (cell to cell) or systemic (long distance), within the plant. We are trying to decipher the type of interaction that prevails between a new isolate of SCMV (SCMV-Mx) and two susceptible (Rogers hybrid GSS 4644 and CIMMYT SL1) and one resistant maize genotype (CIMMYT RL1). The plants were inoculated on the third leaf at the four-leaf development stage. The two susceptible genotypes (Rogers hybrid GSS 4644 and CIMMYT SL1) had infection frequencies of 98% and 75% respectively, whereas the resistant genotype (CIMMYT RL1) never developed symptoms. Symptoms in the susceptible genotypes appeared 3 to 4 days after inoculation as mosaic symptoms on leaf number five, but not on leaves three or four. ELISA tests detected the virus only on leaf five. However, RT-PCR analyses revealed the presence of the genomic viral RNA in all three leaves. This would indicate the presence of either of an undetectable virus titer or a restriction in the assembly of the virion below the systemic symptoms, starting on leaf number five. The absence of symptoms and lack of detection of the virus by ELISA in leaves three and

four, which were fully developed by the time of virus inoculation, might suggest the need for developing leaves for assembly of the virus, but not for replication or movement of its genome. In contrast, no symptoms were observed in the resistant genotype, nor was the virus detected by ELISA at any time on leaves three to five. It was possible to detect the negative genomic viral strand (by RT-PCR), thus implying the allowance of the first stage of replication (i.e., the replication of a sense strand into its corresponding antisense), only in the inoculated leaf. This replicative form could only be detected at short distances from the inoculation site, possibly due to restriction of movement. We are now trying to understand what might be preventing later stages of replication in the resistant genotype, as well as the possible arresting of assembly of the virus in the neighborhood of the infection site in the susceptible genotypes. For this, we will obtain subtracted cDNA libraries to look for genes implicated in the resistant or susceptible response.

Sequence Comparisons of Six Mitochondrial Genomes From Maize and Teosinte

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Plant mitochondrial genomes are unusual in their diversity of structure and rapidity of change. Previously, only six mitochondrial genomes (mtDNA) had been completely sequenced: the liverwort, Marchantia polymorpha, three dicots, Arabidopsis thaliana, Beta vulgaris, Brassica napus, and a monocot, Oryza sativa. We have sequenced another six monocots within the genus Zea which includes fertile (NB and NA genotypes) and male sterile (CMS-T and CMS-C genotypes) maize and two teosintes from section Luxuriantes, Zea luxurians and Zea perennis. In addition, the mtDNA sequences of a closely related grass, Sorghum bicolor, is complete but we are still unsure of the presence of duplications. The sequencing of the Zea mays mitochondrial genomes from NB, NA and CMS-C generated circular maps of 569,630, 701,046 and 739,719 base pairs respectively. CMS-T is 90% finished; Zea luxurians and Zea perennis mtDNAs also generate circular maps which are 539,347 and 570,353 bp long respectively. Sequence comparisons reveal major rearrangements, large duplications, various foreign DNA insertions, but few differences in genPlant mitochondrial genomes are unusual in their diversity of structure and rapidity of change. Previously, only six mitochondrial genomes (mtDNA) had been completely sequenced: the liverwort, Marchantia polymorpha, three dicots, Arabidopsis thaliana, Beta vulgaris, Brassica napus, and a monocot, Oryza sativa. We have sequenced another six monocots within the genus Zea which includes fertile (NB and NA genotypes) and male sterile (CMS-T and CMS-C genotypes) maize and two teosintes from section Luxuriantes, Zea luxurians and Zea perennis. In addition, the mtDNA sequences of a closely related grass, Sorghum bicolor, is complete but we are still unsure of the presence of duplications. The sequencing of the Zea mays mitochondrial genomes from NB, NA and CMS-C generated circular maps of 569,630, 701,046 and 739,719 base pairs respectively. CMS-T is 90% finished; Zea luxurians and Zea perennis mtDNAs also generate circular maps which are 539,347 and 570,353 bp long respectively. Sequence comparisons reveal major rearrangements, large duplications, various foreign DNA insertions, but few differences in gene content. This is the first detailed analysis of sequences and rearrangements in such closely related plant mitochondrial genomes. Annotation data and comparative analysis of the genomes will be presented.

This work has been supported by National Science Foundation grant DBI-0110168.

Long-Oligonucleotide Arrays in Maize for Comprehensive Analysis of Gene Expression

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The Maize Micorarray Project is to provide low-cost, comprehensive, public sector long-oligonucleotide microarrays for gene expression analysis in Zea mays L. The overall objectives are: 1) Produce an array with 70mer oligonucleotides for the >40,000 identifiable unique maize genes which should allow better discrimination among the gene duplications common in maize relative to cDNA arrays and provide a hybridization service, both based on a cost recovery model. 2) Provide a website to distribute microarray information and provide all expression data generated by this project within a project-specific relational database Zeamage, for Zea mays Gene Expression Database, with links to rice and maize genome annotation. 3) Perform expression profiling with a subset of maize tissues to provide a baseline of data and detailed protocols for the community. 4) Utilize the flexible Nimbelgen system to experimentally refine oligonucleotides design for the next generation of 70mer arrays to achieve better discrimination among gene family members and gene duplication.

Current progress to date includes the generation of ~55,000 sequence assemblies from EST, cDNA, and genomic sequences present in Genbank. Oligos optimized for direction of transcription, uniqueness, Tm, and secondary structure criteria have been designed for ~35,000 sequence assemblies with the remaining ~20,000 sequence assemblies in the oligo design phase. The complete 55,000 oligo set printed on two slides is anticipated to be available by May, 2004. A central feature to this project is both a hybridization service available to researchers lacking micorarray facilities and workshops designed to give researchers both the laboratory and statistical training necessary to successfully complete microarray experiments . The first maize oligoarray workshop will be held May 10-15, 2004, in Tucson, AZ. All interested maize researcher are encouraged to apply immediately as space is limited. Details on the workshop and other project specifics are available at http://www.maizearray.org/.

Generating a Pollen Functional Map Using Oat-Maize Addition Lines

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Even though pollen is a rather simple two- or three-celled organism, the construction of a functional male gametophyte requires the coordinated activity of a large pool of genes. Although gene sequences and expression data are rapidly being accumulated, constituting a valuable source of information that should be exploited to integrate genetic maps with functional information, only about 2900 maize EST contigs (TC) out of a total of approximately 27600 have been mapped so far onto existing linkage maps. The recent development of oat-maize addition lines has supplied a shortcut to the creation of maize functional maps, avoiding the identification of sequence polymorphism and the need of large segregating populations. In the present paper we report the use of oat-maize addition lines for mapping 1000 maize pollen EST contigs. The strategy used is based on PCR amplifications performed on DNA derived from oat-maize addition lines corresponding to the 10 maize chromosomes. The analyzed EST contigs, retrieved from the TIGR database, have been selected according to the presence of at least one

EST derived from a pollen cDNA library. Map position was unavailable for all ESTs but one. Using the same standardized PCR conditions for all primer pairs, it was possible to assign about 57% of the TCs to specific maize chromosomes. Important information concerning gene duplication and chromosomal distribution were obtained. Those results represent a good starting point in the construction of a pollen functional map and they will certainly be very useful for the identification of candidate genes underlying mutants affecting viability and functions of the male gametophyte. Furthermore, those results could contribute to the assembly of sequence data, produced in the maize sequencing project, by identifying overlapping BAC clones and resolving alignment conflicts and doubts. Finally, it is currently under development the analysis of radiation hybrid lines obtained from the single oat-maize addition lines, to obtain finer chromosomal localization of the EST contigs.

An Integrated Genetic and Physical Map of the Maize Genome

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With the completion of the rice genome sequencing project in December 2004, the next cereal genome to be sequenced will be maize. The maize genome (~2000Mb) is nearly five times bigger than that of rice (~400 Mb) and is rich in nested retrotransposons, which comprise approximately 80% of the genome. Due to genome size and repetitive DNA content scientists are now performing pilot projects to determine the most efficient strategy to sequence the maize genome. Regardless of the approach, a complete sequence ready physical map of the maize genome will be required. Here we report our finished map of maize genome. A total of 292,039 BACs, equal to 20x genome coverage, have been successfully fingerprinted on high resolution agarose gels using three BAC libraries (Hind III, EcoR I and MboI). These BAC fingerprints were assembled with FPC and anchored to the genetic map . After extensive manual editing, and integrating with near 2,500 genetic markers and 13,688 overgoes, now we have about 2,250 contigs, covering 2358 Mb (equal to 94.3% of the 2500 Mb genome). Of the about 2,250 contigs, 745 of them were genetically anchored with 1930 markers. The 745 anchored contigs covers 1443 Mb, equal to 58% genome coverage. The longest anchored contig is 13.5 Mb in Chromosome 7 while the longest unanchored contig is 10.0 Mb. Of the nearly 1,500 unanchored contigs, about half of them contain less than 10 BAC clones and are about 200 kb in size. By using this integrated physical and genetic map, we have constructed a maize genome duplication map, a maize-rice synteny map, and a maize expression map with the EST-derived overgo markers.

Analysis of Subtractive and Standard cDNA Libraries Produced from mRNA of Drought-Stressed Maize (Zea mays L.) Plants

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Standard and subtractive cDNA libraries were produced from three Mexican maize landraces, Cajete Criollo, Michoacon 21 and 85-2 grown under drought stress conditions. Cajete Criollo and Michoacon 21 are considered to be drought tolerant genotypes whereas 85-2 is susceptible to drought. Leaf water potential, photosynthesis, transpiration rate, soil water potential, relative humidity and vapor pressure deficit were monitored throughout the experiment. Separate subtractive libraries were produced from mRNA from leaves and roots of plants which had reached water potentials between -8 and -11 Bars. The standard library was also obtained from mRNA from a mixture of leaf and root tissue from plants which had reached these water potentials. A subtractive library was produced from mRNA of leaves of plants which had reached water potentials of between -9.8 and -11.4 Bars. A total of 785 clones of average length between 314 and 459 bp were sequenced from the subtractive libraries and 3,367 from the standard library. Clustering was carried out using a CAP3 assembler program and 22 and 116 clusters were identified for the low stress leaf and root libraries respectively whereas 32 clusters were identified for the higher stress leaf library and 2247 clusters for the standard library. Sequences were considered to show a significant match using a cut off E value of 10-6. We identified 5, 13, 21 and 358 clusters respectively that show no homology on comparison to the ZmEST database. 'Gene Ontology' is currently being used to classify the genes according to their putative function.

Gene Expression Patterns during Somatic Embryogenesis in Maize Tissue Culture

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Regeneration in tissue culture is important for improvement of maize using genetic engineering approaches. However, many important maize lines are difficult to regenerate. The program of gene expression during somatic embryogenesis in tissue culture was examined in regeneration-proficient lines in the effort to find key genes that may limit regeneration in more recalcitrant lines. Somatic embryos were generated from eight embryogenic callus lines developed from immature Hi II embryo explants using protocols developed and utilized at the Plant Transformation Facility at Iowa State (http://www.agron.iastate.edu/ptf/web/system.htm). The callus lines were pooled into three groups, and RNA was extracted from samples at 6 time points during incubation under culture conditions that induce embryo formation, maturation and finally germination. RNA was hybridized to 12K gene cDNA microarray chips produced by the Microarray Facility in the Center for Plant Genomics at Iowa State (http://www.plantgenomics.iastate.edu/microarray). The time course analysis of gene expression was conducted using a loop design (Dobbin and Simon 2002) such that each sample is run in triplicate allowing for statistical analysis of the data set.

Identification of Genes Differentially Expressed in Association with SVMV Resistance in Maize by Combining SSH and Macroarray Techniques

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The molecular mechanisms underlying the development and progression of SCMV infection are poorly understood. The identification of differentially expressed genes has been used to recognize candidate genes involved in plant infection processes. In this study, we combined suppression subtractive hybridisation (SSH) and macroarray hybridisation to identify genes whose expression is differently expressed between resistant and susceptible cultivars. Five SSH libraries were constructed using lines F7 and 10940 (BC5 derived SCMV resistant isogenic line from susceptible parent F7 using FAP1360A as donor of resistance genes). 400 cDNA clones from each library were arrayed onto nylon filter membranes. In order to control the quality of cDNA array hybridisations, the sensitivity, linearity and reproducibility of array hybridisations were evaluated. Using TIGR MIDAS and TIGR MEV (http://www.tigr.org/software/tm4/index.html), genes significantly increased or repressed were identified by statistical analysis. After sequencing, 283 genes differentially expressed have been revealed, which account for 10.5% of cDNA clones deposited in the macroarray. Similarity search (http://mips.gsf.de/proj/thal/db/tables/tables_func_frame.html) shows that classified genes are mostly involved in four functional categories (cell rescue, defence, death and ageing; metabolism; signal transduction and transcription). Promising clones from macroarray hybridisation experiments are currently studied in more detail by EST mapping.

Genomic Characterization of the Maize 12-Oxo-Phytodienoic Acid Reductases

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12-oxo-phytodienoic acid reductases (OPRs) are enzymes of the biosynthetic pathway which converts linolenic acid to jasmonic acid (JA). OPRs control the flow of metabolites from the 18-carbon group of Œ±-linolenic acid derivative, 12-oxophytodienoic acid (OPDA) to the 12-carbon group of cyclic Œ±-linolenic acid derivative, 3-oxo-2-(2')(z)-pentenyl)-cyclopentane-1 octanoic acid (OPC) which is the biological precursor of the natural JA. Plants respond to many biotic or abiotic stresses by activation of biosynthesis of JA and its derivatives called jasmonates. These oxylipins serve as signaling molecules to regulate expression of an array of defense-related and developmental genes. The precise role of JA in induction of defense genes is still elusive considering that its precursor, OPDA, is a potent signal as well. However, it has been demonstrated that JA has different regulatory activity from that of OPDA. In our attempt to clearly define physiological function of maize OPRs, we carried out bioinformatics analysis of ESTs representative of more than 280 different Pioneer/DuPont and public cDNA libraries. Using full insert sequencing and 5'RACE cloning we identified several different OPR genes in the maize genome. The number of genes was confirmed by Southern blotting analysis and by using PCR with genomic DNA from oat-maize chromosome addition lines to identify the chromosome location of each individual gene. Genomic structures were predicted for each of the maize OPR genes based on the available public GSS genomic database. Interestingly,

promoters of several maize OPRs contain conserved transcription factor binding elements that have been shown to mediate stress-induced expression of many defense-related genes. Results of RNA profiling by methods including Northern blotting and quantitative RT-PCR indicate that expression of the OPR genes is differentially regulated in non-treated maize organs and in response to infection with fungal pathogens or treatment with defense-associated signaling molecules such as JA, ethylene and SA. This suggests their involvement in pathogen-induced defense responses. By using a reverse genetics strategy, we have identified maize mutants in which function of one of the SA- and Fusarium verticillioides-inducible ZmOPR genes is interrupted by insertions of Mutator transposable elements in its coding sequence. A study is underway to test these mutants for their disease resistance to define this gene's role in defense responses and interactions with pathogens.

Candidate Gene Selection and Molecular Analysis of 50 smk Mutants

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P130

The Robertson's Mutator transposon (Mu) is a powerful engine for mutagenesis in maize. However, in order to harness its full potential for high throughput functional genomics new strategies are needed that facilitate identification of causative insertions in high copy Mutator lines. By working with the pedigreed population, UniformMu, and by efficiently extracting Mu insertion flanking sequences with MuTAIL-PCR, we have developed a high throughput strategy to identify candidate genes using in silico bioinformatics filters and hybridization-based sib subtraction, detailed in another poster. The informatics filter uses recursive BLAST analysis and a clustering algorithm to create a database that integrates sequence annotation, clustering and pedigree information for all mutants in the analysis. The database facilitates identification of candidate genes that contain Mu insertions that are unique to individual libraries as well as Mu insertion sites that are common to two or more mutants that are known or suspected to be alleles. Performed in parallel, the sib subtraction protocol enriches for clones in the library that are linked to the mutant of interest by differential hybridization using probes generated from MuTAIL products from mutant and homozygous wild type sibling plants. Finally, co-segregation of candidate genes with the mutant phenotype is evaluated by PCR genotyping and/or Southern hybridization. Fifty independent confirmed heritable smk mutants were selected for candidate gene analysis. Microlibraries of MuTAIL-PCR products from each line were cloned and sequenced. 384 reads from each line were analyzed in the MuTAIL database (www.UniformMu.org) for bioinformatic analysis and the MuTAIL sequences are deposited in gss at NCBI. Initial in silico subtraction has identified candidate flanking sequences for each mutant. Progress of the candidate gene selection, sib subtraction screen and co-segregation analysis will be presented.

An Integrated Physical, Genetic and EST Map of Maize

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DuPont Crop Genetics

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An integrated genetic and physical map in maize serves as the foundation for positional cloning of genetically mapped genes, and it is essential for genome sequencing. A whole-genome physical map was constructed by high information content fingerprinting (HICF) of a corn BAC library (inbred Mo17). Manual curations, as well as the use of public physical maps (agarose and HICF) and rice synteny, reduced the number of contigs to 3242. Over 140,000 BAC end

sequences were obtained from BAC clones. The low-copy sequences (~16%) provide a rich source of genetic markers and automatically place 6000 genes on the map. Moreover, 18,752 ESTs have been placed on the BAC contigs through overgo hybridization. To integrate the physical map with the genetic map, 2423 genetic markers have been placed on the physical map via association to overgo probes or by extrapolating public markers anchored by PCR. In addtion, thousands of physically anchored EST and BAC end sequences have been mapped using the linkage between diverse maize inbreds. Currently, BAC contigs covering approximately 70% of maize genome have been anchored on the genetic map. With more ESTs being genetically mapped, we expect that contigs representing over 90% of the maize genome will eventually be aligned with the genetic map. The integrated genetic and physical map has been used to map ESTs and traits, and clone mutant genes.

Identification of Genes Coordinately Expressed with eEF1A in Maize Endosperm

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The opaque2 (o2) mutation increases the lysine content of maize endosperm by reducing the synthesis of zein storage proteins and increasing the accumulation of other types of cellular proteins. Elongation factor 1A (eEF1A) is one of the proteins increased by o2, and its concentration is highly correlated with the amount of other lysine-containing proteins in the endosperm. We investigated the basis for this relationship by comparing patterns of gene expression and protein synthesis between a high (Oh51Ao2) and a low (Oh545o2) eEF1A inbred, as well as between high and low eEF1A recombinant inbred lines obtained from their cross. Using an endosperm EST microarray, we identified about 110 genes coordinately regulated with eEF1A. These genes encode proteins involved in several biological structures and processes, including the cytoskeleton, the endoplasmic reticulum and the protein synthesis apparatus. The content of alpha-zein and several cytoskeletal proteins was measured in high and low eEF1A inbred lines, and the levels of these proteins were found to correlate with that of eEF1A. Thus, higher levels of eEF1A in o2 mutants may be related to a more extensive cytoskeletal network surrounding the rough ER and increased synthesis of cytoskeleton-associated proteins, all of which contribute significantly to the lysine content of the endosperm.

High-Throughput Insertional Mutagenesis of Genes Controlling Seed Development

McCarty, Donald R. {1}; Latshaw, Sue {1}; Suzuki, Masaharu {1}; Tan, Bao-Cai {1}; Settles, A. Mark {1}; Koch, Karen E. {1}; Hannah, L. Curt {1}; Messing, Joachim {2}; Larkins, Brian {3}; and Becraft, Phil {4}

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The Endosperm Functional Genomics Project has focused on developing resources for efficient identification and molecular analysis of genes that control seed development in maize. Core resources include the UniformMu inbred transposon tagging population (40,000 M2 and M3 families), 2025 tagged seed mutations, a database of 25,000 MuTAIL Mu flanking sequence tags and a set of 22,000 endosperm ESTs including 2500 full-length cDNA sequences. MuTAIL sequence analysis and candidate gene selection for 110 Mu-stable seed mutants representing diverse phenotypic classes is in progress. Key mutants controlling protein composition (opague16), hormone biosynthesis (vp13, vp15) and aleurone differentiation (widow's peak) processes have been cloned and confirmed using MuTAIL. These forward genetics applications demonstrate a robust set of complementary strategies for identifying the causative insertions in

high copy Mu lines: 1) selection of linked MuTAIL clones by sib-subtraction, 2) selection of candidate clones by in silico subtraction and informatics filtering, and 3) mapping and identification of independent allelic insertions using a blast clustering filter. These strategies are amenable to automation with the two bioinformatics based protocols requiring minimum user input. Additional informatics filters that integrate physical map locations of Mu insertions and cDNA expression data are under development. The MuTAIL database facilitates reverse genetics applications by providing sequence tags that identify random germinal insertions in the inbred UniformMu background including at least 30 knockouts in genes identified by the endosperm cDNAs. These integrated resources facilitate comprehensive forward and reverse genetic analyses of seed development and composition.

A Two Dimensional Proteome Map for Maize Endosperm Development Studies

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{1} INRA; {2} CNRS

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If morphological steps of maize endosperm development are well known, till now, the underlying physiological and molecular mechanisms are largely unknown. One way to appraise these complex processes is the study of endosperm proteome at several key stages through development, which would give an access to the functional gene products and how their expression is modulated. We established a well-annotated reference map of endosperm proteome based on 2D gel electrophoresis of 14 day after pollination endosperm proteins. This stage was chosen because it takes place after cell differentiation and at the beginning of storage compound synthesis. Among the 632 protein spots processed, 454 protein spots were identified, 50 of which matched to two or more proteins with different functions (72% of successful identification), and 178 had no homology to any protein in the NCBInr or ZMtuc-tus databases using the SEQUEST software. 42% of the proteins were identified against maize sequences, 23% against rice sequences and 21% against Arabidopsis sequences. Identified proteins were not only cytoplasmic but also nuclear, mitochondrial or amyloplastic. Proteins involved in metabolic processes, protein destination, protein synthesis, cell rescue, defense, cell death and ageing represent almost half of the 632 proteins analyzed in our study. The most abundant functional category related to metabolic processes (24%). Sixty nine percent of the identified proteins appeared as more than one spot on the 2D gel. At this stage of the analysis, it is not possible to know whether these multiple isoforms correspond to products of different related genes or to post-translational modifications of a same gene product. This proteome map constitutes a powerful tool for physiological studies and is the first step for investigating the maize endosperm development.

Gene Discovery for Maize Seed Composition and Nitrogen Metabolism Traits using the Illinois Protein Strains

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University of Illinois

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Two of the most complex and economically important traits in maize and other cereal crops are endosperm composition and nitrogen use efficiency (NUE). These traits are interdependent on genes acting in both vegetative source and seed sink tissues, thus integrated genetic and physiological studies of whole plant nitrogen metabolism are needed. We are using the unique genetic system provided by the Illinois Protein Strains as the basis for a functional genomics

program aimed at discovering maize genes controlling endosperm composition and NUE. More than a century of divergent selection has generated four populations that span the known phenotypic extremes for grain protein concentration and correlated traits, particularly kernel starch concentration and plant nitrogen metabolism. Inbred lines derived from the Illinois Protein Strains are being used in mRNA expression profiling experiments to identify candidate genes associated with seed composition and NUE traits. Candidate gene are being validated through a combination of physiological and genetic approaches which include: surveying candidate gene allele frequencies in different cycles of the Illinois Protein Strains to test for response to selection, phenotypic characterization of candidate gene mutations in Illinois Protein Strain backgrounds, metabolite profiling of the Illinois Protein Strain inbreds, and tests for association with seed composition and NUE QTLs. We confirm that the alpha-zein genes and the opaque2 transcription factor are important for regulating seed protein concentration. We also have identified asparagine synthetase as a likely candidate gene associated with both high grain protein and increased plant N content. Continued functional genomic comparisons of the Illinois Protein Strains promises to not only identify additional genes controlling seed composition and NUE, but will also provide insights into how the maize genome has responded to long term selection.

Use of an Indica Rice Mutant Collection as a Tool For Root Functional Genomics

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{1} UFPEL; {2} UFRGS

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Rice is currently the major cereal crop in importance, because of its status as a model genome for other crops added to its economic importance. We started a mutant collection in 1999, sponsored by FAO/IAEA, with the goal of generating mutants affecting agronomically important traits. This study has provided a large collection of rice mutants generated at the Center for Genomics and Plant Breeding (CGF) at Federal University of Pelotas (UFPel). Therefore, 321 M3 families of indica rice generated by gamma-ray irradiation from cultivar Taim were characterized for their presence of mutations affecting root morphology. Families showing distinct phenotypes were detected such as absence of seminal roots (asr); root hairness (rh); absence of primary root (apr) and short root (sr). With the completion of the high quality draft sequence of the rice genome, new efforts are now pointed to strategies allowing analysis of a functional genome. Genes involved in root development are poorly described and can serve as models of developmental genetics and stress related problems faced by the roots.

Comparison of Transcription Levels in Immature Ears Between Inbred Lines and Corresponding F1 Hybrid By DNA Microarray Technology

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Millions of hectares throughout the world are cultivated with hybrid crops. However, our understanding of the genetic and molecular causes of the phenomenon of heterosis is still limited. Since a relevant part of the biological regulation occurs at the level of transcription, it is not unreasonable to hypothesise that by monitoring genome-wide expression, it would be possible to gain crucial information on the molecular basis of heterosis. It has been proposed

that hybrid vigour might be due to differences in the expression of sets of genes, functionally related. Therefore, by comparing genome-wide expression profile in the hybrid and in the corresponding inbred parents, it could be possible to identify components of the genetic circuits responsible for heterosis.
Here we present data on transcription profiling in immature ears comparing the two inbred lines B73 and H99 and their corresponding F1 hybrid, produced by DNA microarrays technology, as a contribution to elucidate the molecular mechanisms underlying heterosis. In specific, glass-slide cDNA microarray have been used (University of Arizona, Tucson), each spotted in triplicate with 4905 maize ESTs from 606 immature ear library (R. Schmidt lab, UCSD) plus controls, representing about 2200 different TCs (TIGR Maize Gene Index), corresponding to about 1700 sequences homologous to known genes (plus 300 unknown). Gene expression levels of B73 and H99 inbred lines versus their hybrid have been independently compared, performing 5 replicate hybridizations using fluorescently-labelled cDNA, derived from immature ears. The fluorescence intensity values for microarray spots were acquired and quantified with ScanArray 4000 scanner and QuantArray3.1 software package respectively (Packard BioChip Technologies, Billerica, MA). The resulting data were normalized by using the Lowess and the 50th percentile functions provided in GENESPRING software package (Silicon Genetics, Redwood City, CA - academic evaluation license). Genes whose expression was significantly up- or down-regulated in each experiment were identified by using SAM software (Significance Analysis for Microarrays - Tusher et al., 2001; One class Response, 10-Nearest Neighbor Imputer, Median number of false significant set to less than 1); 60 to 85% of detected ESTs have been confirmed also by GENESPRING confidence gene filtering (t-test, Benjamini and Hockberg FDR multiple-testing corrected).
With respect to hybrid, 63 and 421 ESTs resulted as down-regulated in H99 and B73 respectively, while 169 ESTs in H99 and 851 in B73 resulted as up-regulated. Among all these, 44 down-regulated and 13 up-regulated ESTs were shared between both inbred lines as compared to their hybrid. Even for the genes found statistically significant, the difference in expression level between inbred and F1 is slight, rarely reaching values above the 1.5-fold change. However, if confirmed, such differences in transcriptional activity could have a biological relevance and play a role in establishing heterosis.

Comparative Proteomic Analysis of Maize Silks in Aspergillus flavus Resistant and Susceptible Inbreds

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Research in our laboratory is focused on eliminating aflatoxin contamination in maize (Zea mays L.) by increasing resistance to Aspergillus flavus infection during ear development. Because it has been postulated that the fungus enters the ear via the silks, we are investigating the proteome of silk proteins in maize inbreds that are resistant or susceptible to aflatoxin contamination and/or A. flavus infection. We hope to identify proteins that directly contribute to the resistance phenotype or proteins/genes that can be used for marker-assisted selection in breeding programs. Control silks were collected from Mp313E, Mp420 (resistant), Tx601 (intermediate resistance) and Sc212M, Mp339 (susceptible) 21 days after silk emergence (DAS). Infested ears were inoculated with A. flavus at 15 (DAS) and were collected 21 DAS. Silk proteins were extracted and analyzed by 2-dimensional gel electrophoresis (2-DE). Gel images were analyzed by PDQuest software (BioRad) and comparisons were made among inbreds and between inoculated and uninoculated samples. MALDI-TOF mass spectroscopy and LC/MS/MS

were used to identify common silk proteins and those that consistently differed among resistant and susceptible lines, or inoculated and uninoculated ears. Agar plate assays using GFP-tagged A. flavus were used to study the resistance potential of proteins extracted from the resistant and susceptible genotype.

Oat-Maize Addition and Radiation Hybrid Lines: Development and Application

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All ten different maize chromosomes are represented as individual chromosome additions among 50 fertile disomic oat-maize addition lines (2n = 6x+2 = 44) except for maize chromosome 10 that was recovered as fertile disomic short arm telocentric line (2n = 6x+2t = 42+t+t) only. Most disomic addition lines were developed from *Seneca 60* maize as chromosome donor and *Starter Sun II*, and *GAF/Park* oats as chromosome recipients. The transmission behavior of the addition chromosomes was characterized by cytological and molecular means. In a recent series of experiments maize chromosomes from inbreds *B73* and *Mo17* were recovered as single additions to haploid oat, and offspring are being produced to establish more fertile addition lines of maize chromosomes of different genetic origin. Fertile monosomic additions (2n = 6x+1 = 43) - the foundation for radiation hybrid (RH) lines - were generated by crossing disomic oat-maize additions back to their parental oats. Monosomic addition BC1-seeds were treated with 20-50 krad dosages of gamma rays to induce as many breaks as possible in the added maize chromosome, and BC1F2-offspring scored for transmitted maize deficiencies and oat-maize translocations to develop the RH lines. PCR-based markers were allocated to the RH lines by a presence versus absence test to define the breakpoints by markers with known map coordinates. The physical sizes of the diminutive maize chromosomes were visualized by genomic in situ hybridization of labeled genomic maize DNA to chromosomes from meristem cells of the corresponding RH lines. To date, panels of RH lines for maize chromosomes 1, 2, 3, 4, and 6 have been produced for physical marker mapping with low to medium resolution based on the statistical distribution of the breaks along the appropriate maize chromosome arms. The panel of chromosome 9 RH lines, presently, allows a physical marker mapping at a medium to high resolution of approximately 5 Mbp distance. We recently have been optimizing the panels for high resolution mapping for chromosomes 2, 4, and 6. Low to medium resolution panels currently are being developed for chromosomes 5, 8, and the short arm of chromosome 10. This material is based upon work supported by the National Science Foundation under Grant No. 011134.

Transcriptome and Proteome Wide Analysis of Crown Root Initiation in Maize

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Plant roots serve important functions among which water and nutrient uptake and anchorage are the most important ones. The complex root system of maize consists of the embryonic primary root and seminal roots, and an extensive postembryonic, shoot-borne root system composed of crown- and brace-roots which make up the major backbone of the root system. Crown roots are formed at consecutive underground stem nodes, while brace roots are formed late in development from aboveground stem nodes. The coleoptilar node is the first stem node that is formed early during seedling development.

The monogenic recessive mutant rtcs (rootless concerning the crown and lateral seminal roots) completely lacks all shootborne roots including crown-, brace- and seminal roots. The mutant rtcs is affected at an early stage of root development since for none of the missing root types primordia are initiated (Hetz et al., 1996). In this study gene expression and protein accumulation profiles of wild-type and rtcs coleoptilar nodes were compared. Transcriptome analyses with unigene microarrays containing 12,000 different maize cDNAs as well as proteome analyses which compared differential 2D-protein profiles which were subsequently and analyzed differentially accumulated proteins via MALDI-ToF mass spectrometry were carried out. The results will be presented and discussed.

Optimizing Conditions for SNP Detection Using Oligonucleotide Microarrays

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Oligonucleotide microarrays have the potential to provide a high-throughput format for mapping and mutation detection. We have used a trial SNP array containing oligonucleotide probes designed to detect SNPs present between B73 and Mo17. The oligonucleotide probes were varied in length and in the position of the SNP base within the probe. This array was then interrogated with genomic DNA and pooled PCR products using different hybridization buffers and varying hybridization temperatures. The combination of temperature and probe length is critical for optimizing the discrimination between SNPs. The use of 3M betaine in the hybridization buffer resulted in 1.5-3.2X higher discrimination. This dataset is extremely useful in planning the array design for future microarrays and for determining the appropriate hybridization parameters for maximizing the discrimination between SNPs. We are currently in the process of applying these conditions for the design and hybridization of mutagenized DNA samples in order to detect novel EMS induced mutations.

Status of the Maize TILLING Project

Weil, Clifford {1}; Till, Brad {2}; Monde, Rita-Ann {1}; Marshall, Kelly {1}; Boys, Kathryn {1}; Loesch-Fries, Michael {1}; Hsia, An-Ping {3}; Schnable, Pat {3}; Greene, Elizabeth {2}; and Henikoff, Steven {2}
{1} Purdue University; {2} Fred Hutchison Cancer Research Center; {3} Iowa State University

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Targeting Induced Limited Lesions IN Genomes (TILLING) is a reverse genetics technique that allows screening of large mutant populations for single base changes in a target sequence of interest. Once identified, the mutations will be sequenced and users will then be able to request maize seed containing the mutations for further study. As part of a National Science Foundation Plant Genome Project, we are offering a TILLING service to the maize community using the inbred lines W22 and B73. Details about the service, costs, deliverables and instructions on how to place your orders will be presented.

Functional Genomics to Understand Chloroplast Gene Regulation

Wostrikoff, Katia {1}; Brenchley, Jamie {1}; Harris, Faith {1}; Kikis, Elise {1}; Belcher, Susan {2}; Walker, Nigel {2}; Osbourne, Erin {2}; Barkan, Alice {2}; and Stern, David {1}
{1} Boyce Thompson Institute for Plant Research; {2} University of Oregon

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A collection of ~2,200 transposon-induced maize mutants with defective chloroplasts was generated (refer to talk on the Photosynthesis Mutant Library (PML) collection and see project

website (http://chloroplast.uoregon.edu)). Approximately 1,000 mutants which still contained some chlorophyll, yet exhibited specific or global defects in the thylakoid photosynthetic complexes, ATP synthase and/or Rubisco were screened for chloroplast gene expression defects at the protein level using antibodies to subunits of the photosynthetic apparatus. This protein data was used as a guide to assess RNA accumulation and maturation using probes for each chloroplast gene encoding a subunit of the missing photosynthetic complex(es). 44 mutants were found with RNA defects that fell into 22 classes; these ranged from strong reduction or lack of monocistronic transcripts to overaccumulation of some transcript forms, altered processing accompanied by the absence of normal processing intermediates, and the appearance of new transcripts. While 18 of 22 mutant classes were novel and thus may lead to the characterization of new nuclear genes required for chloroplast gene expression, previously observed RNA phenotypes were also found, allowing the recovery of additional mutant alleles. In one case, similarity in the RNA phenotype to mutants characterized in Arabidopsis and Chlamydomonas led to the identification of two mutant alleles of the conserved HCF107 nuclear gene, whose product is required for the expression of the psbB-psbT-psbH-petB-petD polycistron. An example of a novel mutant is crp4, which fails to accumulate the cytochrome b6f complex and exhibits an aberrant petD transcript pattern. The crp4 mutant may be defective in a ribonuclease activity required for normal petD mRNA maturation. We also analyzed nine mutants which were strongly affected in Rubisco accumulation. Among these mutants, we have focused on one mutant that is deficient in Rubisco and photosystem II, and one mutant that is deficient solely in Rubisco. These mutants may define new factors required for Rubisco synthesis and/or assembly. The PML collection is also useful for reverse genetics, carried out through PCR-based screening using the Mu transposon tag. This approach allowed us to isolate an insertional allele of ZmSIG2, a gene which encodes a sigma factor targeted to both mitochondria and chloroplasts and presumed to have a role in transcription initiation. Taken together, analysis of the PML collection is leading to greater insights into transcriptional and post-transcriptional processes in the chloroplast.

Global Expression Analyses of Genes Involved in Meristem Organization and Leaf Initiation

Zhang, Xiaolin {1}; Buckner, Brent {2}; Janick-Buckner, Diane {2}; Nettleton, Dan {3}; Schnable, Patrick {3}; Timmermans, Marja {4}; and Scanlon, Mike {1}
{1} University of Georgia; {2} Truman State University; {3} Iowa State University; {4} Cold Spring Harbor Laboratory

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All above ground organs of higher plants are ultimately derived from specialized organogenic structures called shoot apical meristems (SAMs). The SAM exhibits distinctive structural organization, marked by tissue zonation and cell layering. The structure of plant SAMs is correlated with their function, such that new leaves are initiated from the peripheral zone of the SAM and the central zone replenishes new meristematic cells that are lost during organogenesis. Experiments are proposed to identify and analyze genes required for meristem function and early stages of leaf development in maize. Laser dissection microscopy is a powerful technique that permits the isolation of RNA from specific cell types within fixed plant tissues. RNA collected from 1,000-10,000 cells is sufficient for use in microarray analyses of global gene expression. The relatively large size of the maize vegetative meristem, approximately 250 meristematic cells are recruited into the incipient maize leaf, renders this plant especially tractable for this experimental system. The laser microdissection/microarray technique is being used to capture cells from specific domains of the maize meristem and

newly-formed leaf primordia for use in comparative analyses of global gene expression. The differential expression patterns of candidate genes will be verified by real time RT-PCR and in situ hybridization) of transcript accumulation in maize tissues. These experiments will microdissect gene expression patterns in meristems and leaf primordia, and promise to provide novel insight into mechanisms of plant development.

The Challenge Program for Unlocking Genetic Diversity in Crops for the Resource-Poor
Ziegler, Robert
Kansas State University / UGD Challenge Program
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This poster provides a summary of the Challenge Program's mission and five subprograms.

Quantitative Traits & Breeding Posters

Towards the Detailed Analysis of QTLs for Southern Leaf Blight and Gray Leaf Spot Resistance
Balint-Kurti, Peter; and Brewster, Vickie
USDA-ARS, North Carolina State University
P146
Quantitative resistance is the dominant form of resistance utilized in cultivated maize and QTLs for resistance to several diseases have been identified. However, almost nothing is known about the molecular genetic or phenotypic basis of quantitative resistance in maize or any other crop. Working with the foliar diseases Gray Leaf Spot and Southern Leaf Blight, we are identifying and mapping new sources of resistance from diverse germplasm. In addition, we are developing materials and methods for the detailed characterization and fine-mapping of selected QTLs. We will report our progress on the development and assessment of controlled environment screens, the development of near-isogenic lines and the preliminary results of our germplasm screening. Approaches for candidate gene analysis will be discussed.

Identification of QTLs Controlling Ustilago maydis Resistance in Two Recombinant Inbred Populations
Baumgarten, Andrew; Suresh, Jayanti; May, Georgiana; and Phillips, Ronald L.
University of Minnesota
P147
We report the identification of QTLs contributing to Ustilago maydis (corn smut) resistance in two populations of recombinant inbred lines. These two populations were generated by crossing a susceptible inbred (CMV3) to two inbred lines (A188 and W23). Resistance to U. maydis infection was highly heritable (h2 = 0.82-0.93) in both RI populations. Several RI lines segregated for U. maydis resistance within specific tissues of the plant. A genetic map consisting of approximately 90 SSR loci was constructed for each RI population and used to detect QTLs for U. maydis resistance. Strong QTLs (LOD scores ranging 4.0 to 9.0) contributing to the frequency and severity of U. maydis infection were detected in both populations and explained 55% to 62% of the variation in U. maydis infection. Similar regions of chromosome two, four, and nine were found to significantly contribute to U. maydis resistance in both RI populations. Furthermore, many of these QTLs were shown to contribute significantly to U. maydis resistance within specific plant tissues, such as the tassel or ear. Digenic epistatic interactions between detected QTLs were found to significantly affect the U. maydis resistance within each of the two RI populations. Similarly, significant QTL by environment interactions

were detected in both populations, resulting in one case where the effect of a QTL for U. maydis infection was reversed between two environments. Finally, CAPs markers were designed from previously described resistance gene analogs (RGAs; Collins et al. 1998) and mapped onto the two RI populations. A CAPs marker designed from the RGA probe pic17 showed strong association with a QTL contributing to U. maydis resistance on chromosome two.

Population Genetic Analysis of Candidate Genes for Variation in Sorghum Panicle Architecture

Brown, Patrick {1}; Rooney, William {2}; Vollbrecht, Erik {3}; Kresovich, Stephen {1}
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The five races of domesticated Sorghum bicolor (bicolor, guinea, caudatum, kafir, and durra) show exceptional variation in panicle architecture. Since wild sorghum panicles are comparatively uniform, the morphological diversity seen in cultivated types may be the result of human selection detectable through population genetic tests. Candidate genes from maize and rice were cloned in sorghum and sequenced in a small panel of sorghum inbreds, landraces, and wild accessions. The sequence data is being used to conduct tests of neutrality and to generate gene-specific polymorphic markers for subsequent linkage and association mapping.

Our characterization of inflorescence architecture in sorghum has been designed for explicit comparison with a similar effort underway in maize, as reflected in our choice of candidate genes and phenotyping criteria. Patterns of nucleotide diversity in wild and domesticated conspecifics will be compared between sorghum, an inbreeder, and maize, an outcrosser. Comparative expression profiling between the perfect-flowered sorghum panicle and the two maize inflorescences, tassel and ear, should help clarify the evolutionary and developmental mechanisms of monoecy. Future manipulation of inflorescence architecture in cereals has the potential to improve traits as diverse as harvest index, grain quality, flowering time, and resistance to pathogens.

The comparative approach described here is especially promising for a crop like sorghum, which is a close relative of maize (~12.5 million years of divergence) that receives comparatively little US research funding. Sorghum is the world's fifth most important cereal and is an important subsistence crop throughout much of the semi-arid tropics. With its small, diploid genome (750 Mbp), sorghum is the ideal bridge between maize and other panicoid cereals of global importance such as sugarcane (Saccharum) and pearl millet (Pennisetum).

QTL Analysis of Stomatal Density

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University of Missouri – Columbia

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Stomatal pore size, controlled by guard cells, is regulated by dynamic changes in the intracellar concentrations of inorganic and organic ions and soluble sugars. Stomatal closure is important in reducing transpirational loss. It is one of several mechanisms for maintaining water potential such as growing deeper roots, leaf angling, and leaf rolling. Several genes involved in stomatal closure have been cloned in *Arabidopsis thaliana* and *Vicia faba*. Some examples are the *KAT1* and *KAT2* inwardly rectifying K+ channels, the *GORK* outwardly rectifying K+ channel, sulfonylurea glibenclamiden insensitive *AtMRP5*, and the extracellular Ca2+ sensor *CAS*. Drought is a major limitation to global crop production. Understanding stomatal closure is key to our goal of comprehending the genetic mechanism by which maize plants tolerate water stress. A subset of ninety-four mapping lines from the IBM population and parents B73 and Mo17 were

grown in the greenhouse to the 7-leaf stage. This population has increased resolution due to random intermating for 3 generations. Epidermal cast samples were taken from leaf 5 using cosmetic lacquer. Stomate counts per square centimeter were taken. Stomatal data was averaged across five replicates. A subset of 251 loci evenly spaced throughout the chromosomes were used for QTL analysis. QTL analysis was conducted using QTL Cartographer version 1.16. Stomatal density was correlated with plant architecture measurements taken after water stress as part of a separate experiment using PROC CORR in SAS. Candidate genes corresponding to QTL regions were identified using the maize bins maps.

A Comprehensive Study of Genetic Structure in a Collection of American and European Maize Inbred Lines and Its Use in Association Genetics

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We defined a collection of 372 maize inbred lines, representative of most of the genetic diversity of European and North-American breeding material and that also includes a subset of important inbred lines from southern or central America. This sample was chosen based on pedigree information, and recent data on molecular analysis of ancestral population varieties from both continents (Rebourg et al., 2003, Theor Appl Genet, 106:895-903 ; Dubreuil et al., 2003, 45th annual maize genetic conference) e. It includes first cycle inbred lines directly derived from these ancestral populations, as well as more recent inbred lines, which are supposed to have accumulated favorable alleles. A set of 67 SSR distributed genomewise displayed an average number of 10.5 alleles per locus and allowed us to study the genetic diversity and the structure of the collection. Three different statistical methods were used: distance-based clustering, Principal Component Analysis, and a model based structure analysis using software STRUCTURE.

This last approach showed that the collection is structured in eight groups. It makes it possible to estimate, for each individual, the proportion of its genome originating from each group. These groups can be described as Northern-Flint, European Flint, Tropical, miscellaneous Dent, Stiff Stalk, Lancaster1, Lancaster2 and Pop-Corn. European Flint has been generated by the hybridization between Tropical and Northern Flint material. The first five groups appear highly consistent with results mentioned above on the analysis of ancestral population varieties. On the other hand, Stiff Stalk and Lancaster groups result from recent selection within the American Dent pool.

Such a genetic structure must be taken into account in association genetics, in order to avoid spurious associations due to genomewise linkage disequilibrium. Flowering time was measured for the inbred line panel using a multi-local experiment.. Simultaneously, the panel was genotyped for an insertion/deletion in Dwarf8 gene, which showed an association with flowering time in a former study by Thornsberry et al. (2001). We studied the genetic association between phenotypic and genetic data using several statistical methods that include the individual genetic characterization provided by STRUCTURE. These statistical approaches, including linear and logistic regression, gave comparable results and confirm, using a broader set of material, the association revealed by Thornsberry et al. (2001).

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Identification of Key Genes for Drought Tolerance in Tropical Maize

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In field-grown maize (Zea mays), losses in grain yield are maximal when drought occurs during the flowering stage. Genetic studies in maize showed that yield components and morphological traits involved in drought tolerance process are regulated by several genes (Ribaut et al., 2002). Knowledge of the genes underlying the drought tolerance quantitative trait loci (QTLs) would be extremely useful both for the understanding of the biological basis of tolerance and for utilization in further breeding for increased drought tolerance.

We want to develop new molecular-based approaches that will complement conventional breeding practices used for improvement of drought tolerance in corn. Our main goal is to assemble a 'toolkit' of genes that are correlated with drought tolerance, and which can be assayed by simple techniques in breeding programs to evaluate germplasm for drought tolerance or susceptibility. In addition, we are interested in positioning the identified genes on the genetic map for use in marker assisted selection, and for the construction of the consensus map of loci involved in drought tolerance in corn.

Sucrose regulation, through anabolism and perhaps even more importantly catabolism, is a major component for drought tolerance. In sink organs, sucrose is cleaved by sucrose synthase to produce UDP-glucose and fructose, or it is hydrolysed by invertase to yield glucose and fructose, which are then used as energetic and structural sources for multiple biosynthetic pathways (Lunn, 2003) and starch biosynthesis. Differential expression of regulatory genes involved in the carbohydrate pathway have been reported in the literature. Water stress environments inhibit acid invertase activity, and Zinselmeier et al. (1999) have shown that genes involved in the starch biosynthetic pathway are coordinately regulated under stress and a decrease in the expression of these genes causes a loss of starch and embryo abortion.

Thus, much of our recent efforts have been focused on genes of the carbohydrate pathway. We are assaying expression of candidate genes by quantitative RT-PCR and microarray expression analysis experiments in tolerant and susceptible lines as well in recombinant inbred lines. To complement the expression studies of carbohydrate genes, the activities of key enzymes of the carbohydrate pathway and the concentration of their substrates or products have been quantified. In agreement with published results using US germplasm, we have found that increased expression of cell wall invertase is correlated with drought tolerance in tropical germplasm

Identification of Quantitative Trait Loci Controlling Early Flowering of a Northern Flint Maize Inbred Line

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In order to expand maize cultivation area in Hokkaido region of Japan where the growing season length is limited by cool climate, it is necessary to breed the superior early flowering hybrid with high yield and resistance to lodging. Northern Flint inbred lines derived from local varieties in Hokkaido shows early flowering and cold tolerance. However, their yield and lodging

resistance in hybrid combinations are less than those of medium-late flowering Dent inbred lines. In addition, lack of superior early flowering Dent inbred line is an obstacle of developing early hybrids. Molecular marker of early flowering genes is considered to be an effective tool to convert medium-late flowering inbred lines to early ones using Northern Flint inbred lines as a donor of the genes. The candidate gene (CG) approach has been applied in plant genetics for characterization and cloning of quantitative trait loci (QTL). If the genes related to QTL of the flowering time are identified, the Genetic Diagnosis of the maize flowering time will be possible. In this poster, relationship of QTL of the flowering time with the CGs that may influence to them was analyzed.
One hundred fifty F2:3 lines derived from the cross of inbred lines P1 and P2 were grown at NARCH, Sapporo (N43, E141) in 2003. P1 belonging to Dent is late flowering in Hokkaido region, and P2 belonging to Northern Flint is early flowering. The variations between the parents were investigated in four genes, D8, An1, Vp1, and CK2, and the markers that can discriminate parents' genotype of each gene were made. Mapmaker/EXE and Mapmaker/QTL performed mapping and QTL analysis based on 150 plants of the F2:3 lines, using 110 SSR markers and the four gene markers. LOD threshold was 2.0.
The full length map was 1287 cM, and average distance between neighborhood markers was 12.4 cM. Eight QTLs of growing degree day (GDD) to silking and 6 QTLs of GDD to anthesis were identified in chr.1, chr.3, chr.4, chr.7, chr.8, and chr.9. The genes, D8, An1, Vp1, and CK2, were located in chr.1, chr.1, chr.3, and chr.1. One QTL related to silking date was located between D8 and An1. In the future, we will analyze the relationship of the QTL with the variation of D8 and An1. Furthermore, exploration for the additional candidate genes related to early flowering of Northern Flint is continued referring rice and arabidopsis genome information.
This work is supported by KAKENHI (15780008)

The Genetic Basis of Recurrent Selection Gains for Maysin in Maize Silks
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P153
We report the identification of QTLs contributing to Ustilago maydis (corn smut) resistance in two populations of recombinant inbred lines. These two populations were generated by crossing a susceptible inbred (CMV3) to two inbred lines (A188 and W23). Resistance to U. maydis infection was highly heritable (h2 = 0.82-0.93) in both RI populations. Several RI lines segregated for U. maydis resistance within specific tissues of the plant. A genetic map consisting of approximately 90 SSR loci was constructed for each RI population and used to detect QTLs for U. maydis resistance. Strong QTLs (LOD scores ranging 4.0 to 9.0) contributing to the frequency and severity of U. maydis infection were detected in both populations and explained 55% to 62% of the variation in U. maydis infection. Similar regions of chromosome two, four, and nine were found to significantly contribute to U. maydis resistance in both RI populations. Furthermore, many of these QTLs were shown to contribute significantly to U. maydis resistance within specific plant tissues, such as the tassel or ear. Digenic epistatic interactions between detected QTLs were found to significantly affect the U. maydis resistance within each of the two RI populations. Similarly, significant QTL by environment interactions were detected in both populations, resulting in one case where the effect of a QTL for U. maydis infection was reversed between two environments. Finally, CAPs markers were designed from previously described resistance gene analogs (RGAs; Collins et al. 1998) and mapped onto the two RI populations. A CAPs marker designed from the RGA probe pic17 showed strong association with a QTL contributing to U. maydis resistance on chromosome two.

QTL Analysis for Maize Weevil Resistance in Tropical Maize

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The maize weevil (MW), Sitophilus zeamais (Motsch.) is an important pest of stored maize (Zea mays, L.), which causes severe losses in tropical agroecologies. Several studies have established the polygenic inheritance of MW resistance, the importance of maternal effects, and the additive and non-additive gene actions. In this study, we analyzed quantitative trait loci (QTL) associated with resistance to MW in tropical maize (Population 28). A total of 163 F2 genotypes derived from the cross CML290 (susceptible) x Muneng-8128C0HC1-18-2-1-1 (resistant), 61 restriction fragment length polymorphism (RFLP), and 90 simple sequence repeat (SSR) markers loci were used for the QTL analysis. Resistance to MW was evaluated using four susceptibility parameters under artificial bioassays. Grain damage (GD), grain weight losses (GWL), adult progeny (AP), and Dobie index (DI) were evaluated on grain from F3:4 lines in three replications across two environments. The method of composite interval mapping (CIM) was used for detection and identification of QTL. Five QTL on chromosomes 4, 5, 6 (2 QTL), and 9 explained 24% of the phenotypic variance (ó2p) and 43% of the genotypic variance (ó2g) for GD. Eight QTL dispersed across the genome were determinated to affect GWL and explained 22.5% of ó2p and 47%of ó2g. Nine QTL (31% of ó2p and 90% of ó2g) were identified for ID and four (12% ó2p and 27%of ó2g) for AP. Four main areas for MW resistance were identified in bin 1.05, 6.00/1, 6.05, and 9.05, with 6.01 accounting for the most phenotypic variation. These regions map in areas know to carry genes associated with cell wall biochemistry, disease and insect resistance. Both parents contributed resistance alleles. Genetic effects of QTL for maize weevil resistance were mainly dominant for GD, DI and AP, while additive effects were found for GWL. QTL x environmental interaction was found to be significant for half of the QTL detected. This indicates that environment has a large influence on the expression of MW resistance. Since the identified QTL only account for half of ó2g and less than a third of ó2p, several major and minor QTL remain undetected using this narrow-based mapping population. Future mapping efforts should use lines that show greater phenotypic variation for MW resistance under several environments to identify robust markers for marker assistance selection (MAS). Given the time consuming nature of evaluating maize for resistance to MW, MAS holds considerable promise in accelerating the incorporation of MW resistance into elite maize varieties in the future.

Identification of QTL Associated with Root Architecture Under Well-Watered, and Water-Stressed Conditions in Zea mays

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Drought alone contributes 17% to the average annual yield loss in maize (*Zea mays*). It is the single most significant environmental obstacle to improving grain yield. Many physiological traits have been linked to drought resistance including osmotic adjustment, anthesis-silking interval, leaf surface area and root architecture. QTL associated with root architecture play an integral role in drought resistance by facilitating the uptake of water from sources deep below the soil line. Our goal was to identify the QTL involved in root architecture in *Zea mays* under well-watered, and water-stress conditions. Two QTL experiments were performed one well-watered

and one water-stressed. In the well-watered experiment, a subset of 94 mapping lines from the intermated B73 x Mo17 (IBM) population was planted in five reps in a randomized complete block design. The seed was sown in a peat based growth medium and the plants were grown in a greenhouse under well-watered conditions for two weeks. After the two-week period, shoot mass, root branching, primary root length, seminal root number and root mass were measure for each plant. The water-stressed experiment was conducted in the same manner. To prevent immediate desiccation of the plants a polyacrylamide water retainer was added to the growth media. After the initial two week, well-watered period the plants were allowed to grow without water for ten days. At the end of the 10 days the same traits were measured along with the relative water content of the 4th leaf. Genotypic data for 251 markers, evenly distributed throughout the maize genome, were used to construct a genetic map with Mapmaker Exp version 3.0 for Unix. QTL analysis was performed by using QTL Cartographer version 1.16. 32 total QTL were identified, 20 for the well-watered traits, and 12 for the water-stressed traits. The 20 QTL identified in the well-watered experiment accounted for 74%, 38%, 77% and 81% of the phenotypic variance in primary root length, root branching, seminal root number and shoot mass respectively. The 12 QTL identified in the water-stressed experiment accounted for 25%, 13.5%, 41.2%, 17.2%, of the phenotypic variance in primary root length, root branching, seminal root number and root mass. QTL identified were compared to previous QTL experiments. Many of the well-watered QTL correspond to QTL regions previously identified in other root architecture studies. Novel QTL for root growth under water-stressed were identified in bins 3.05, 8.01, 10.01 and 10.05. Candidate genes were selected for the QTL using the maize bin map.

How Is the TraitMill™ Platform Delivering Valuable Target Genes for Cereal Breeding?

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P156

Although steady yield improvements have been achieved since decades through conventional breeding and improved agricultural practices, it is expected that plant productivity will be further stepped up by applying breakthrough biotechnology tools. In that respect, genetic engineering is generally considered to be very challenging for improving complex and quantitative traits such as yield. Moreover, predicting the potential agronomic value of transgenes involved in yield components remains difficult. To meet this challenge, CropDesign's TraitMill biotechnology platform allows assessing, in a controlled greenhouse environment, the effect of plant-based transgenes and novel alleles on agronomically valuable traits such as growth rate, organ size and architecture, seed yield and harvest index. TraitMill is thus designed to identify new targets for crop improvement and to deliver comprehensive and quantitative data on individual gene/allele effects. The current focus of the platform is on rice, a globally important crop and a good model for other important cereals such as corn and wheat. Key genes modulating yield components of rice, grown either under non-stress or stress conditions, have been identified and confirmed in two or more subsequent plant generations. Major high-throughput technologies used in the platform, such as super-efficient rice transformation protocol using Gateway binary vectors and imaging/data analysis, will be presented. For rice, some examples of novel single gene alleles with significant positive effect on grain yield (increased by over 50%), biomass (about 30%) and harvest index (30-60%) will be presented together with some novel insights in plant growth and genetic engineering-based crop improvement. These results not only point to the clear impact that single genes and novel alleles can have on quantitative traits, but also emphasize the relevance of genetic engineering approaches for improving yield

and yield stability components and for generating additional genetic diversity. Importantly, the interest of these results and technology tools for corn and corn improvement will be discussed.

Comparison of "Z" Lines With "PL" Open-Pollinated Inbred Lines of Maize, With Initial Inbreeding of One Half

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In a previous theoretical research it was demonstrated that Z lines and inbred open-pollinated (OP)lines, derived from a population of non inbred unrelated invididuals, had the same inbreeding values in coincident generations. This was true even as the formulae to calculate inbreeding were different. The explanation for this result is that OP lines were arithmetical multiples of the Z lines. It was remarked, however, that OP lines had higher genotypic diversity than the Z lines. In the inbreeding equation of these lines terms of selfing and plant-to-plant crosses were involved. Thus, in practice it was impossible to derive them through successive generations. It was postulated that if pollination whithin a line was randomly made then it was possible to continue the derivation of the Z and OP lines in the field, and to calculate their inbreeding in successive generations. In the present study, using as a base population a series of inbred lines of one generation of selfing, the same phenomenon of the previous study takes place. As in the inbreeding equations obtained in the present study there are only terms that include selfing, there is no problem in the calculation of inbreeding in successive generations.

Identification of QTLs For Heterosis Using Two Pseudo-Backcross Populations in the B73 x H99 Background

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The exploitation of heterosis in plant breeding is considered one of the most revolutionary advancement in plant improvement. However, despite a long story of successes, there is still a striking gap between the extensive agricultural practice of hybrid vigour utilization and our understanding of the cause of such phenomenon. As a contribution to gain some understanding of the heterotic phenomenon in maize, and to shed light on the genetic mechanisms underlying it, we are following an integrated approach comprising both molecular and quantitative analyses. Here we present results derived from QTL analyses for yield and several plant traits utilizing two pseudo-backcrosses. In particular, a population of 142 recombinant inbred lines (RILs), obtained from the original cross B73 x H99 after 13 generation of selfing, was used for the production of three pseudo-back cross (PBC) populations by crossing each of the 142 RIL with both the original parental lines (PBC-P1 and -P2) and their F1 hybrid (PBC-F1). In addition, a tester population (TC) was also produced, by crossing each of the 142 RIL with tester line Mo17. Owing to the mating design adopted, the mapping population of reference is the B73 x H99 RILs. This population has been characterized for 158 molecular markers, mainly Simple Sequence Repeats. Our genetic map covers 2087.3 cM, with an average distance between adjacent loci of 13.2 cM. All the populations described (i.e. RILs per se, PBC-P1, PBC-2, PBC-F1 and TC, for a total of 716 genotypes) were grown in three locations (2 reps per location).

Data for vegetative growth, plant cycle and yield were collected and analyzed. The underlying QTLs were identified by Composite Interval Mapping as implemented in PLABQTL (Utz and Melchinger, 1996) and QTL Cartographer (Basten et al, 1999), with significance thresholds determined by a permutation test. Analyses across all populations allowed us to identify loci displaying dominance and/or overdominance, and to estimate their possible contribution to heterosis. We have identified QTLs explaining heterotic variation for all the characters considered. Some QTLs were in common between traits. Among the significant QTLs, 9 were selected for further analysis. To speed up the process of producing Near Isogenic Lines for such QTLs, we started with the identification in the recombinant inbred lines at F5 generation (RILsF5) of individual plants with a heterozygous state in the chromosomal regions containing the QTL(s) of interest. Once identified, each RILF5 plant has been crossed with the corresponding RILF13. Our goal is to produce two independent sets of three NILs for each of the selected QTLs.

Agronomic Performance of Maize Populations Developed by Different Crop Management and Selection Schemes

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Selection methodologies applied to local populations and in situ conservation of genetic diversity are by definition two paradoxical strategies. Participatory plant breeding has been proposed as the way in which these approaches can be addressed. The objective of the research work was to compare four populations developed by different management and selection procedures under the participatory crop improvement approach. A set of 25 families were randomly selected from each of four populations for evaluation in 2003: 1) A local adapted population (LP) from Jag√ºey de Ferniza, Saltillo, Coah., obtained by farmers through traditional mass selection; 2) The first generation of the local population (G1LP), obtained through a seed production scheme, using detasseled rows as females; 3) and 4) Two populations generated by the combination of local and improved germplasm, representing the first cycle selected for early (EIP) and late (LIP) maturities, respectively. Half sib families represented the first two populations, whereas in the last two, full sib families were obtained. Field evaluations were conducted at two locations: El Prado, N.L. (1890 masl) and Jag√ºey de Ferniza, Saltillo, Coah. (2100 masl), being the irrigated and rain fed environments, respectively. Results showed significant differences (P,â§0.01) among populations for most recorded traits; the performance across environments was similar due to the unusual rainfall occurred during the cycle at the rain fed environment, which made a similar genetic expression on the two environments. A canonical discriminant analysis showed an overlap on the individual evaluated families; however, populations were characterized and grouped by sets of correlated traits in both environments. Populations LP and G1LP were comparatively more diverse than EIP and LIP as an effect of the selection methodology. The relative magnitude on the difference was highly evident (P,â§0.01) between the local population (LP) and the improved populations (EIP and LIP), as a contribution of the improved germplasm to the locally adapted population. Even though, there was not a significant difference between the locally adapted population and the seed production scheme at the rain fed location, there was a difference on ear yielding of 6.4%; whereas, the difference between LP and EIP populations was in the order of 18.7%. The main difference between LP and G1LP populations is that, on the seed production method selfing within individuals was eliminated, which may contribute to the divergence in magnitude among subsequent generations. Populations were differentiated by the first component, which was

characterized by days to flowering, associated to moisture content; the second component discriminated the populations by ear yielding, positive correlated with ears per plant, and negative associated with plant and ear height. In summary, populations showed differences on the agronomic performance, which are directly related to the procedure and selection criteria evaluated. The contribution of the improved germplasm to the local adapted material was shown on ear yielding, nevertheless, yield has not been the most important trait used as selection criteria, where a selection index was instead applied.

Breeding Effect of Selection at the Level of Haploid Sporophyte in Maize

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The main objective of this study was to evaluate the efficiency of selection for quantitative traits in maize (Zea mays L.) at the level of maternal haploids. Two different experiments were conducted.

In the first experiment, haploids from the population SA were selected for plant traits and crossed with a tester line (MK-01y). In the second experiment, haploids from the population SP were crossed with a haploid-inducing line (MHI) as a tester, followed by selection for ear traits. Both in the first experiment, on the basis of plant traits, and in the second one, on the basis of ear traits, haploids were classed into three groups according to the values of these traits.

Testing the resulting hybrids obtained from three groups of haploids in both experiments revealed that selection among haploids for several plant and ear traits had a major effect on these traits in the hybrid plants. The high-yielding hybrids were those derived from haploids with the largest ear trait values. The same correlation was found for the other plant traits.

In the second experiment, by crossing with the haploid-inducing line as a tester along with the hybrid seeds of each of the three groups selected, seeds with haploid embryos were obtained. The haploids derived from the haploids of the selected group were compared. The investigated haploids differed significantly in three ear traits. As with hybrid testing, the values of haploids of the first group produced from the largest ears are note worthy. The first group of haploids (10.12cm) significantly surpassed the second (8.83 cm) and the third (9.05 cm) in ear length. The ear diameter of the first group haploids (2.07 cm) also significant exceeded that of the second (1.75 cm) and that of the third (1.82 cm). The mean number of floret rows per ear was 16.2 in the first group of haploids, 14.2 in the second and 15.0 in the third.

The progeny control at the level of hybrid plants conducted in both experiments as well as the progeny control at the level of haploid plants in the second experiment revealed that the phenotype of the selected haploid plants was due to their genotype. So it can be concluded that selection on a haploid level is possible and effective. Moreover, it was shown that non-allelic gene effects (additive and epistatic) selected for at the haploid level can have a significant contribution on trait values of diploid hybrid plants.

Utilization of Maize Haploid Plants in Recurrent Selection Procedure

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Maize (Zea mays L.) maternal haploid plants were used to improve two synthetic populations SP and SA. Three cycles of Haploid Sib Recurrent Selection (Chalyk, Rotarenco; 1999) were

conducted for each population. The specific feature of this recurrent slection is that selection is conducted at the level of haploid plants. The key advantage of selection among haploids is that haploids lack allelic gene interactions (dominance, overdominance), thus enabling more efficient selection of favorable genes with non-allelic effects (additive and epistatic).
The gain per selection cycle in grain productivity ranged from 1.5% to 17.8% in different experiments and at different locations.
Along with diploid synthetic populations of different selection cycles haploids obtained from them were compared. As a result of the performed selection, not only did the diploid plants of the populations improve, but so did the haploids obtained from them. Improvement of haploids relative to traits such as ear length, ear diameter, plant height, and leaf length, was fully comparable to the improvement of the diploid plants. Comparison of the diploid and haploid plants in the improved populations permits the suggestion that the investigated diploid populations improve mostly by an increase in the frequency of genes with non-allelic effects.
Estimation of combining ability of the investigated populations was performed. For this purpose the following interpopulation crossings were made: SPC0 x SAC0, SPC1 x SAC1, SPC2 x SAC2, SPC3 x SAC3. A distinct increase in grain productivity was observed during crossing of synthetics of the third selection cycle. The gain per selection cycle in grain productivity in the hybrids was 2.6%.
Overall, it can be concluded from this work that selection at the level of haploid plants is an effective means to improve synthetic populations per se and their combining ability. Moreover, maternal haploid plants can be used for a general evaluation of the contribution of non-allelic gene effects in the development of traits of interest to the breeder.

Identification of Quantitative Trait Loci (QTLs) Conferring Resistance To Mal de Rayo Cuarto (MRC) Virus in Maize

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Mal de Rayo Cuarto is a major viral disease of maize (Zea mays L.) in Argentina that may produce severe grain yield losses under favorable environmental conditions on susceptible genotypes. The causal agent, Mal de RÃ–o Cuarto Virus, is transmitted by Delphacodes kuscheli Fennah. Breeding for resistance is considered the most adequate strategy to control this pathology in maize. The aim of this work was to characterize the phenotypic disease response under natural infestations, and the molecular marker patterns of maize genotypes for mapping Quantitative Trait Loci of resistance to Mal de Rayo Cuarto. A F2 population of 221 individuals was genotyped with simple-sequence repeats markers and their progenies F3 were assessed for disease response for three years in the endemic region (Sampacho, CÃ“rdoba, Argentina). Disease scoring included separate ratings for symptoms at individual F3 plants; plant height was an additional trait evaluated. Plant height average, and incidence, severity and intensity of symptoms were calculated at F3 family level. Quantitative Trait Loci mapping analysis permitted to locate Quantitative Trait Loci to the short (QTL 1) and large (QTL 2) arms of chromosome 1, and chromosomes 4 (QTL 3), 8 (QTL 4) and 10 (QTL 5) related to the disease symptoms, and plant height. Plant height was used as “a trait supporting MRC resistance QTL” because dwarfism is a major MRC symptom. QTL 1 was co-localized with QTL/genes conferring resistance to Maize Streak Virus, while QTL 3 and QTL 5 were located to similar positions to QTL/genes for resistance to High Plains Virus and Wheat Streak Mosaic Virus. There are not previous reports for virus resistance genes at the positions of QTL 2 and QTL 4. The application of Quantitative Trait Loci mapped both in the present and in future

works, after independent validations, will become more powerful the conventional breeding for resistance to Mal de Rayo Cuarto.

Root-ABA1, a QTL Affecting Root Angle and Lodging, Leaf ABA Concentration and Other Traits in Maize

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A major QTL affecting leaf ABA concentration (L-ABA) was mapped on bin 2.04 in Os420 x IABO78 (TAG 97: 744-755). Following five backcrosses and two subsequent selfings, nearly isogenic backcross-derived lines (BDLs) homozygous for the (+/+) or (_/_) L-ABA QTL alleles were obtained for both parental genetic backgrounds. The (+/+) and (_/_) BDLs and hybrids obtained by factorial crossing between the Os420 and IABO78 BDLs were evaluated at two water regimes over three years. The QTL significantly affected L-ABA, RWC, stomatal conductance, yield and root lodging. Additionally, the QTL significantly affected the brace root angle and the diameter of the brace roots. Due to its effects on root traits and L-ABA, the QTL has been named *root-ABA1*. The positional cloning of *root-ABA1* is being attempted starting from a large F_2 population (ca. 1,000 plants) produced by crossing Os420 (the parental line contributing the + allele) and the Os420 (-/-) BDL. By molecular-marker analysis, we plan to produce segmental BDLs families homozygous for different crossover events at *root-ABA1*. Segmental BDLs will be tested for fine mapping and to ascertain to what extent the effects on L-ABA and root traits might be due to one or more linked QTLs. Based on the results of the fine mapping and by exploiting the maize-rice synteny maps, we will try identifying a maize physical chromosome region carrying the gene/s responsible for *root-ABA1*. A microarray-based gene expression analysis is also ongoing using tissue samples from contrasting (+/+) and (-/-) pairs of BDLs taken at various developmental phases and stress conditions.

Heterosis and Combining Ability of CIMMYT and NARS Lines

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Exotic germplasm is oftenly used by NARS(National Agricultural Research System) as source germplasm or for better utilization of hybrid vigor. Making crosses between lines with different heterotic background (exotic and indigenous) accelerates hybrid development. Heterosis and combining ability of maize inbreds is of great value to maize breeders for hybrid development.

Considering this fact, twenty CIMMYT inbred lines were used to cross with four inbred from NARS India. The lines were divided into two sets L1to L10(Set 1) and L11 to L20(Set 2). Lines from each set were crossed with four testers in line x tester fashion to produce 40 crosses in each set. Therefore, two trials were constituted with 54 entries that include 10 CIMMYT inbreds, 4 NARS inbreds and 40 resultant crosses. Both sets were evaluated over three diverse agroclimatic conditions.

Highly significant differences in grain yield were observed for lines, testers while non-significant for line x tester interaction. In both set, the top five yielding hybrids that also showed high heterosis, had yield ranging from 4.61 to 5.02 t/ha. In first set, inbred L1(0.23t/ha) and L5(0.26t/ha) were best general combiner for grain yield. L7 x T4, L9 x T4 and L2 x T2 were the best hybrids with a yield of 4.8t/ha, 4.46t/ha and 4.44t/ha, respectively. These three hybrids also had the positive and significant SCA effect for grain yield. In second set, L12(0.31t/ha),

L13(0.31t/ha), L17(0.71t/ha) and L20(0.16t/ha) were best general combiner for grain yield. The three highest yielding hybrids were L17 X T4, L20 X T4 and L12 x T2 with a yield of 5.02t/ha, 4.69t/ha and 4.66t/ha, respectively. Out of three only one had positive and significant SCA effect for grain yield. Among testers T2(0.12t/ha) and T4(0.16t/ha) showed positive and significant GCA for yield. It is also evident that three of five top yielding hybrids involved T4 as the tester parents. Only lines L5, L13, L20 and tester T4 showed the stable performance across the environment. Among best hybrids, L2 x T2, L9 x T4, L12 x T2 and L20 x T4 had stable performance across for grain yield.

Results from this study once again highlighted the importance of exotic germplasm in NARS system. Exotic lines with good GCA effect for grain yield over environment could be ideal source for future breeding. Superior and stable hybrids could be tested in large area and more number of locations for its release. In future, more number of NARS lines could be crossed to agronomically superior exotic lines to harvest more heterosis.

Quantitative Trait Locus Mapping of Agronomically Important Traits in Maize with Near Isogenic Lines

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Near isogenic lines (NILs) are a valuable resource for genome mapping and QTL identification. A set of 89 NILs (TBBC3) was developed from maize lines B73 and Tx303 using Tx303 as the donor parent and B73 as the recurrent parent for quantitative trait locus (QTL) analysis. Three generations of backcrossing to B73 followed by a generation of self-pollination were used to generate each NIL. Hybrids were generated between the TBBC3 lines and the Mo17 maize line. In an effort to represent an introgressed region of each chromosome arm from Tx303 in the B73 background, marker assisted selection (MAS) for Tx303 alleles was performed at 18 restriction fragment length polymorphism (RFLP) loci distributed throughout the genome. Simple sequence repeat (SSR) markers were used to identify the boundaries of each Tx303 introgression and determine the relative proportion of Tx303 introgressed into B73 for each NIL. A linkage map was created from a B73 x Tx303 F2 population using SSRs and the RFLP markers used in MAS to generate the TBBC3 NILs to serve as a framework to define the locations and boundaries of Tx03 introgressions. To identify QTL for developmental and agronomic traits, the TBBC3 NILs, Tx303, B73, and Mo17 were planted in a split-plot design with three replications. B73 x Mo17 and the TBBC3 x Mo17 hybrids were grown at four locations in a ten by ten lattice design with two replications. Both experiments were grown in the summers of 2001 and 2002. Phenotypic information was gathered for days to anthesis, days to silking and anthesis-silk interval (ASI) in each experiment. Several QTL were detected for days to anthesis, days to silking and ASI in the inbred and hybrid experiments after comparing phenotypes of a set of NILs or NIL x Mo17 hybrids containing a common Tx303 introgression to B73 or B73 x Mo17, respectively, using the ESTIMATE statement of PROC MIXED in SAS. These results demonstrate that the use of NILs is a viable approach for QTL mapping in maize. Seeds of the NIL stocks and their marker genotypes will be made freely available upon request to J.B. Holland.

Historical and Biological Bases of the Concept of Heterotic Patterns in 'Corn Belt Dent'
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P166
In 2004 the concept of heterotic patterns is fundamental to hybrid maize breeding theory and practice in the USA. As hybrids increase in importance in maize in other regions and in other crop species, plant breeders apply the lessons of Corn Belt Dent (CBD) heterotic patterns. However, the origin and development of the concept of CBD heterotic patterns have not been critically examined. CBD heterotic patterns were created by breeders, and are not the result of historical or geographical contingencies. The concept of heterotic patterns developed in the 1960s and 1970s. Academic interest in heterotic patterns increased in the late 1980s, stimulated by the availability of DNA based markers and attempts at using markers to identify heterotic patterns. For CBD open-pollinated varieties and first cycle inbreds it would not have been possible to identify heterotic groups using molecular markers, had they been available. CBD heterotic patterns were created by breeders through trial and error from a single race of corn. Applying the concept of heterotic groups in a hybrid breeding program will result in divergent heterotic groups.

Transposable Element Posters

Diagnosis of Hot Spots for Mu Integration in the Maize Genome: A Progress Report
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P167
Excellent examples of how nuclear cofactors can influence the insertion site distribution of active transposable elements (TE) were published last year. In one case, the reconstructed vertebrate TE Sleeping Beauty exploits interactions with a common DNA-binding protein of the high mobility group class (Ivics, et al., 2003), and the yeast retrotransposon Ty5 integrase is tethered by interaction with heterochromatin protein Sir4p (Zhu, et al., 2003). We predict that similar cofactors influence Mutator (Mu) insertion site distribution in the maize genome, based on cumulative experience with PCR-based reverse genetics (TUSC). We presented a model on Mu insertion hot-spots at MGC 2003, discussing a clear forward bias for transposition to defined sites within genes - particularly 5' UTR and proximal promoter regions. Both the somatic and germinal programs of Mu transposition appear to share an equivalent insertion bias, suggesting that cut-and-paste and replicative mechanisms of transposition respond to similar influences. We postulate that these influences are exerted by interactions between Mu transposase and host-encoded nuclear cofactor(s), supported by data from electrophoretic mobility shift assays (EMSA). We have conducted further EMSA, yeast-1-hybrid screens, and proteomics experiments to help gain insight into the identities of those proteins specifically binding at a well-characterized Mu insertion hot spot found in the 5' UTR of the empty pericarp-2 locus (emp2). Current findings and their implications for the model will be presented.

Establishing an Ac/Ds-Based Enhancer Detection and Gene Trap System in Maize

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The Ac/Ds transposon family has been succefully used for the establishment of a enhancer detection/gene trap strategy in Arabidopsis thaliana but not in maize. To this aim we will use pGS1, a construction designed by the group of Hugo Dooner that includes the BRONZE (Bz) gene interrupted by the transposon itself and C1 located inside the sequence of the transposon and under control of the CaMV35S promoter. To design the enhancer detector element, we have modified pGS1 to include uidA as a reporter gene inserted within the Ds element in front of a minimal CaMV35S promoter. Within the T-DNA border we have included a Wx marker gene under the control of the maize UBI promoter. We are currently generating parental homozygous transformant lines that contain the modified pGS1 construct in genetic backgrounds lacking Ac transposase activity. Parental crosses of Ds lines with a stable transposase source linked to Wx will produce four phenotypes in the F2 generation: 1) Kernels expressing Wx, indicating that Ac is present, 2) Kernels expressing Bz, C1, and Wx genes, indicating both that there was a linked Ds transposition or that the transposase is still present, 3) Kernels expressing C1 and Wx genes, indicating no Ds transposition, and 4) Kernels expressing Bz and C1, indicating a stable unlinked Ds transposition event. The establisment of this strategy will allow us to directly select transformed plants through phenotypic traits of kernels and generate a collection of enhancer detector lines available for the scientific community.

Manipulation of Lycopene-b-cyclase in Maize

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We have recently cloned the Pink Scutellum (Ps1) gene in maize which encodes the lycopene b-cyclase using an Ac mutagenesis strategy. Lycopene b-cyclase is required in seed and seedling tissues for the production of both xanthophylls and abscisic acid (ABA). The disruption of this gene results in the accumulation of lycopene in maize embryos and the precocious germination of kernels. One of the primary advantages of an Ac insertional mutagenesis, is the ability to generate multiple unstable insertion alleles. To date, we have identified 9 independent insertions from 400 transposition events. Among these insertions, we obtained alleles that condition both strong and weak mutant phenotypes. Excisions of Ac can also generate novel alleles through the creation of 'footprint' alleles. Duplications or deletions of sequences immediately flanking Ac can give rise to stable new alleles with subtle or dramatic gene function changes. We are using HPLC analysis to characterize the accumulation of carotenoid in Ps1 footprint alleles in comparison with near-isogenic parental lines. The goal of the project is to identify the functionally important domains of lycopene b-cyclase and to manipulate the accumulation of carotenoids in maize kernels.

Development of a Two-Component Activator/Dissociation Tagging System in Maize

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Ac offers a number of advantages as an insertional mutagen such as low copy number in the genome, low forward mutation rate and a tendency to transpose to closely linked sites. We are currently distributing and fine-mapping the transposable element throughout the maize genome for use in regional mutagenesis experiments (http://bti.cornell.edu/Brutnell_lab2/BMGG_home.html). To date, approximately 50 near-isogenic lines have been generated that contain a single active Ac element at a precisely defined site in the maize genome. These Ac's can now be used as launch pads to mutagenize regions flanking these Ac insertions. One of the disadvantages of utilizing Ac is that because Ac is unstable, lines must be continuously genotyped to ensure that the element has not transposed to a new locus. The use of a two-component Ac/Ds system would circumvent this problem. Ds elements are stable derivatives of Ac that transpose only in the presence of Ac. Therefore, it is possible to mutagenize a gene of interest with Ds and then render it stable by segregating the transposase source away. Conversely, it is still possible to get reversion of the mutation and to create footprint alleles by crossing it back to an Ac transposase source.

The cloning and characterization of an immobilized derivative Ac (Ac::im) containing a twelve base pair deletion at the 5' end of the element will be presented. This Ac::im provides a stable source of transposase for use in a two-component Ac/Ds tagging system. Through genetic analysis Ac::im has been shown to display the typical negative dosage effect of Ac. This Ac has been maintained in a uniform W22 inbred background and we have not detected any germinal transposition events in approximately 2500 kernels examined. Most importantly, Ac::im has been shown to mediate excision of the Ds element present in r-sc:m3 at an average frequency of 4.4%. Sixty-five percent of these newly transposed Ds elements can be detected through Southern blot analysis. We present evidence that the majority of Ac::im-mediated Ds excision occurs during gametophytic development.

The lack of mapped Ds elements throughout the maize genome is the major disadvantage of a two component Ac/Ds tagging system. Currently, we are developing techniques to distribute and map Ds elements through the genome. Preliminary results of these techniques will be presented. Finally, to test the efficiency of a two-component system, we have initiated a regional mutagenesis using the Ds present at the R locus as a donor and Ac::im as a source of transposase. Approximately 300 Ds excisions have been selected and will be self-pollinated to create segregating families. Lines will be examined for mutant phenotypes that segregate with a newly transposed Ds.

Establishing an Activation Tagging System in Maize (Zea mays L.) Through a Modified Suppresor/Mutator Mobile Element

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We are implementing a first activation tagging system suitable for the identification of dominant (gain of function) mutations in Zea mays L. Our main goal is to generate a transposant plant

population for the maize scientific community and study genes involved in reproductive development. The genetic construct used to perform plant transformation is derived from the Spm/dSpm transposon family and includes an immobile source of transposase that is under the control of the CaMV35S promoter, a mobile non-autonomous element comprising a tetramer of this same promoter, and 2 selectable markers to identify stable unlinked transpositions: the bar gene conferring resistance to the herbicide BASTA and Su1 that converts the pro-herbicide R7402 (DuPont) into sulfonylurea (herbicide), which inhibits plant growth. We are using plants lacking Spm transposase activity as genetic background to produce parental transposant lines, which will be subsequently crossed to lines showing transposase activity to produce new transpositions events that eventually result in dominant mutations. As an initial step, we have tested a tissue culture regeneration protocol in three experimental genotypes (O'Connor-Sanchez et al. 2002 Plant Cell Rep 21: 302-312), and performed several transformation experiments through biolistics using embryogenic-organogenic callus derived from shoot tips of in vitro germinated seedlings. A first group of transposant lines have been generated from the first transformation experiments after in vitro selection. In vivo selection will be performed with selectable gene markers bar and Su1 after plant regeneration. To ensure the recovery of transformants containing single stable insertions, alternative experiments are being conducted using Agrobacterium tumefaciens as a vector for transformation.

The Capsid of a Novel Maize Retrotransposon Interacts with Light Chain 8, a Protein with Diverse Roles in Molecular Trafficking

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A large and unique lineage of plant Ty1/copia retrotransposons (called Sireviruses) is found in diverse plant species. Founding members of this group include SIRE1 from soybean, Endovir from Arabidopsis, ToRTL1 from tomato, and Opie-2 and Prem-2 from maize. We have observed two unique features common to this group of retrotransposons. First, all elements seem to have an extended gag gene in comparison to other Ty1/copia retrotransposons. Gag proteins typically assemble into virus-like particles, which are obligate intermediates in retrotransposition. Second, many Sireviruses encode an extra open reading frame after pol, referred to as an env-like ORF. Envelope proteins are typically found in retroviruses and mediate infectivity. In the Sireviruses, however, the actual function of the extended Gag and the env-like ORF are both unknown. In an effort to understand function, a two-hybrid screen was used to identify maize proteins that interact with the Gag extension of a maize Sirevirus. A strong interaction was observed with the maize homologue of Light Chain 8 (LC8). Homologous rice retrotransposon Gag extensions also interact with LC8, suggesting that this interaction is preserved evolutionarily.

LC8 is a highly conserved protein in animals, plants and fungi and was originally identified as a light chain component of the dynein microtubule motor complex in Chlamydomonas. In plants, LC8 is unlikely a part of the dynein mictrotubule motor, as no other components of the dynein motor are present in flowering plants. LC8, however, is associated with a number of protein complexes and also interacts with several animal viruses. For some of these associations, LC8 is thought to serve as a docking protein that facilitates intracellular trafficking of various molecular cargos. Current research is focusing on the universality of Gag-LC8 interactions within the Sirevirus group and on understanding some of the functions of LC8 in plants. These experiments should shed light on why Gag proteins from the Sireviruses interact with LC8 and the significance of this interaction for retrotransposition.

Regional Activator (Ac) Mutagenesis in Maize

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The primary objective of this project is to distribute the transposable element Activator throughout the maize genome for use in regional mutagenesis programs (http://bti.cornell.edu/Brutnell_lab2/BMGG_home.html). Our goal is to generate a series of near-isogenic lines each harboring a single active Ac at a well-defined genetic position for regional mutagenesis. Our genetic analysis has been directed at uniformly distributing Ac elements throughout the maize genome utilizing a Ds reporter and by exploiting the negative dosage effect of Ac. DNA flanking 72 active Ac elements has now been cloned and sequenced. DNA flanking 22 of these Ac elements has been too repetitive for us to generate single copy probes for use in RFLP analysis. Thus, to date 50 Ac elements have been positioned on one of three publicly available recombinant inbred populations. Ac's have been mapped to each of the 10 chromosomes and 18 of the 20 chromosome arms are represented by a least one insertion allele.

To explore the potential and limitations of Ac insertional mutagenesis, a non-directed mutagenesis of chromosome 1S has been initiated. We selected several thousand transposition events and self-pollinated ears to create 1921 F2 ears that are segregating a unique Ac together with the donor Ac. As the genetic position of each of the donor Ac's has been determined, we will also be able to assay the relationship between insertion site and genetic distance. Three phenotypic screens are being conducted on the 1921 lines. Prior to shelling, each ear was examined to identify obvious defective kernel phenotypes. Sandbench screens are being used to identify embryo defective mutants and mutant seedling phenotypes that appear to segregate as a single recessive trait. Because the population has been maintained in a uniform W22 inbred background , screens for fairly subtle changes in phenotypes are permitted as the families are extremely uniform. Finally, field screens will be conducted to identify mature plant mutations.

Work will be presented on the preliminary characterization of a mutation that was identified in sandbench screens as a recessive wilted seedling. DNA blot analysis was used to show that the mutant phenotype is linked to a novel Ac insertion. Using an inverse PCR protocol we have cloned DNA flanking this Ac insertion and have begun detailed phenotypic characterizations of the mutant.

Discovery of Helitron Type Transposable Elements in Maize

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We recently described a maize mutant caused by an insertion of a Helitron type transposable element (Lal et al., 2003). We have now searched the maize genome sequences and discovered three other Helitrons. These bear sequence similarity to the maize mutant sh2-7527 insertion only at the termini. These elements, ranging up to approximately 36kb in length, are embedded with copies of retroelements and pseudogenes. Based on the comparison of the insertion site and PCR amplified genomic sequences, these elements inserted between AT dinucleotide sequence. A blastn analysis of both the 5' and 3' termini along with the flanking

non-Helitron sequences produced more than 100 hits. All were derived from maize sequences. Sequence analysis indicated that these hits represent terminal ends of the other members of the Helitron family which were inserted throughout the maize genome. We demonstrate that Helitrons are abundant and continue to play an important role in the evolution of the maize genome. In addition, the Helitrons we have discovered bear distinct features that are unique to maize. For example, the sequences similar to replication protein A (RPA) found in Helitrons from other species are completely absent in the three Helitrons, we discovered in maize. We also report transduction of an intact portion of a cytochrome P450 monooxygenase gene by a maize element. Our data provide strong evidence that Helitron type transposable elements are active in the modern maize genome.

Epigenetic Modification of Mu Activity

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The Mutator system of transposons is regulated by the MuDR class of elements, which encode two genes, mudrA, the putative transposase, and mudrB, a helper gene. The mudrA gene is sufficient to cause excisions of reporter elements in the absence of mudrB, but mudrB is required for new insertions. We have isolated deletion derivatives that express only the mudrA (d201) or the mudrB (d112) genes. We have made lines that carry both derivatives and have found that they fail to compliment with respect to new insertions. This suggests that one or both derivative has lost some functionality, or that the two genes must be present in cis to be functional. Since we MuDR can show strong position effects, and d201 is at a new position and has only weak and variable somatic activity, it is also possible that this derivative is less functional because of a position effect. Indeed, we have found that in a mop1 mutant background d201 shows a pronounced increase in somatic activity (more heavily spotted kernels). mop1is a mutation that was isolated in the Chandler lab that affects both silenced MuDR elements and paramutable alleles. We are currently testing d201 alone and in combination with d112 in a mop1 mutant background to see if alleviation of epigenetic silencing of d201 can result in the appearance of new insertions either with or without an additional source of mudrB. We are also investigating a MuDR element that shows a strong position effect. MuDR(p3) is a duplicate copy of the original MuDR isolate, MuDR(p1). It shows a pronounced reduction in somatic excisions, but a similar frequency of new insertions as MuDR(p1). We show that MuDR(p3) cannot reactivate an epigenetically silenced MuDR(p1) element, that it expresses mudrA and mudrB at roughly the same level as MuDR(p1) and that duplications of MuDR(p3) are invariably associated with increased levels of somatic excision of a reporter element. We also show that MuDR(p3) can be silenced by Mu killer, and that the mop1 mutation does not affect the somatic excisions caused by this element or its duplication frequency. We have some evidence that MuDR(p3) shows an increased frequency of early reversions. We are currently exploring the possibility that MuDR(p3) is associated with changes in the resolution of double-stranded gaps introduced by Mu excision at various times during somatic development.

Taming the Mutator System

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Over the past few years a large number of Mu-induced mutations have been made available to the maize genetics community. Of course, getting the mutations is the easy part; isolating the insertion is more difficult. Much of that difficulty stems from the presence of large numbers of actively transposing Mu elements in lines in which the mutations were originally isolated. We have developed a genetic system for taming the Mutator system. Using this system, it is possible to reliably eliminate Mu activity in F1 plants, making isolation of individual insertions considerably easier. The system can also be used to rapidly identify suppressible alleles. The strategy employs a stock that carries a single dominant locus, Mu killer, that can reliably and heritably silence multiple MuDR elements in a single generation. After silencing, activity in the form of a single MuDR element can be reintroduced in a controlled fashion.

Retrotransposon Activation in Nascent Polyploids and Silencing in Subsequent Generations

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Retrotransposons occupy more than 40% of the human genome and up to 80% of some grass genomes amongst of which Long Terminal Repeat (LTR) retroelements are the most abundant. While most retrotransposons are often considered as an inert component of the host genome, some were shown to be transcriptionally active under specific stress conditions like biotic and a-biotic stresses such as wounding, oxidative stress, pathogen infection or microbial elicitors. In a recent work we showed that the wheat Wis2-1A retroelement was transcriptionally activated in the first generation of a newly-synthesized polyploid. Furthermore readout activity from the element LTRs lead to silencing or activation of adjacent genes.

In this study we extend these observations by following the course of Wis2-1A expression during two more generations. We confirmed that Wis2-1A was highly expressed in the first amphiploid generation (S1) while silent in its diploid parents. In addition we found that in subsequent self-pollinating generations, the second (S2) and third (S3) generation, Wis2-1A was silent. In addition, we tested the expression of two Wis2-1A adjacent sequences located in opposite direction to the readout transcription from the 3'LTR. These genes, namely the leaf specific Thionin-like gene and the Puroindoline-like gene were previously shown to be silenced in the first generation of the synthetic amphiploid. We show here that they regained their activity in the subsequent S2 and S3 generations...,

This work shows that a nascent amphiploid undergoes rapid fluctuation in retrotransposon activity, namely activation in the first generation and silencing in the next. Similarly the silencing of retroelement-adjacent genes is reversible and correlates with the element activity status. These data suggest that the first generation upon formation of a new polyploid species is facing the strongest genomic shock as reflected by transposon activation and by the relatively high levels of plant sterility. Interestingly, fertility increases in S2 and S3, in association with transposon silencing and the restoring of normal gene activity. The ability to rapidly 'absorb' the genomic shock of polyploidy is probably essential for the establishment of nascent polyploids as new species.

Initial Results from Analysis of the RescueMu Transposon-Tagging Gene Discovery Strategy in Maize and Continuing Research

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Because the large size and high preponderance of repetitive DNA in the maize genome makes a straight-forward sequencing and gene discovery approach difficult, the Maize Gene Discovery Project (MGDP) utilized two different complementary strategies: targeted RescueMu transposon insertion mutagenesis and EST sequencing. In most instances, a single RescueMu male founder taken from a transpositionally active line was used in crossing to create planted grids of up to 48 rows X 48 columns. All rows and usually 4 columns were sequenced in a grid. This setup helped in the characterization of probable germinal insertions: a row and column match or multiple recoveries of the same plasmid across the grid. Library plates are made for each grid to save all instances of insertions within rows and/or columns. PCR screening of grid library plates enables a researcher to search for gene(s) by verifying a row and column match

specifying the grid address (plant) where a probable germinal insertion occurred. Library plates may be ordered from http://www.Mutransposon.org/cgi-bin/order.cgi and seed from the Maize Genetics Cooperation Stock Center (http://www.uiuc.edu/ph/www/maize).

Initial results from the first 6 RescueMu grids have yielded 9.23 MB of maize DNA containing ~13,166 distinct loci originating from the sequencing of 31,320 non-parental plasmids. ~8,740 genic loci or ~66% were characterized by comparison to maize and plant ESTs, proteins, or GENSCAN prediction of genes. All 10 maize chromosomes have RescueMu insertions into genes. Computational and manual identification of RescueMu GSS contig data has verified that there are some Mu insertion 'hotspots' (identical trRescueMu insertion sites among independent grids). However, although a bias for insertion into G+C-enriched sequence was noted, there does not seem to be strong support for any preferential insertion site motifs. The creation of a 9-bp target sequence duplication (TSD) was found to occur in most instances of Mu transposition. Data also suggest that some significant fraction of the single recovery class may include probable germinal insertions that were missed due to insufficient sequencing depth. Conversely, some insertion events found in more than three rows are most likely pre-meiotic events.

The extensive data collected from the MGDP will be utilized to create Agilent arrays containing 60-mer oligos to ~22,000 maize elements (arrays with 44,000 elements will be used later). These arrays will be used for transcriptome profiling and will incorporate probes for gene families, gene-specific elements, and alternative splicing products in tassel ESTs. Gene family trees based on synonymous substitutions will be subsequently mapped onto biochemical pathways and compared with resulting microarray data. This data will be used ultimately to (1) begin to study gene expression during the sporophyte to gametophyte transition and (2) to look at evolutionary histories of genes expressed during this developmental stage and the fates of duplicated genes.

Transposon-Induced Deletions: A New Tool for Plant Genomics Research

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Since their discovery by McClintock, transposable elements have been associated with the generation of a variety of genome rearrangements, including deletions, direct and inverted duplications, and translocations. Some of these rearrangements may result from homologous recombination between copies of transposons at non-allelic positions; in this case, transposon sequences provide dispersed sequence homologies for ectopic recombination. In contrast to this 'passive' role for transposons, we have identified an alternative transposition mechanism by which transposons can play an active role in genome evolution.

This mechanism involves transposition reactions which utilize the termini of different elements. When transposase acts upon the two ends of a single transposon, the element moves around the genome. Whereas, when transposase acts upon the two ends of different elements, the genome 'moves' around the transposon. This can generate a variety of major chromosomal rearrangements. We show that in maize, a pair of Ac termini in reversed orientation and separated by 13 kb can undergo transposition reactions resulting in inversion, deletion, and other local rearrangements. In each of these cases, the rearrangement breakpoints are bounded by the characteristic footprint or target site duplications typical of Ac transposition reactions. These results show how transposition reactions involving reversed transposon ends could contribute significantly to genome evolution by generating deletions, inversions,

duplications, and other rearrangements, and by creating new genes through shuffling of coding and regulatory sequences.
We are attempting to utilize these alternative transposition pathways for the efficient production of interstitial deletions in maize. Our system utilizes a transgene construct containing maize Ac/Ds transposon ends in tandem or reversed orientation within a I/dSpm transposon (Ned1; Nested deletions 1). The Ned1 construct has been transformed into maize; subsequent crosses have introduced the En/Spm transposase to mobilize Ned1 to various genomic locations, and the Ac transposase to activate the deletion process. The action of Ac transposase on the Ac termini within Ned1 is expected to generate an unlimited set of nested deletions with one end anchored at the transgene locus. The Ned1 construct contains marker genes for detection of both Ned1 transpositions and Ac-induced deletions, as well as sequences for easy cloning of deletion endpoints via plasmid rescue. Results of this project will be presented.
This approach could be extended to the production of a set of maize lines containing Ned1 elements at dispersed sites throughout the genome. Researchers could then use these lines to isolate deletions and other rearrangements in specific regions of the genome for a variety of research applications, including 1) to map molecular and genetic markers to defined intervals; 2) to assign functions to individual gene copies within complex loci; 3) to determine the effects of gene copy number on expression and silencing; 4) to provide landmarks for unambiguous alignment of YAC or BAC clones which may contain multiple repetitive sequence elements.
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Activation Tagging for Rust Resistance in Maize and Barley

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Obligate fungal pathogens of plants, such as rusts, interact specifically with their host plant causing changes in host gene expression. Potentially over-expression mutants of a host gene could prevent the growth of the rust pathogen leading to disease resistance. Such mutants can be generated and recovered by activation tagging.
A chimeric Ds transposon consisting of 250bp of both terminal inverted repeats of Ac , two maize ubiqitin promoters in both orientations and a GUS reporter gene was constructed:

{5'AcTIR..<-ubi ubi-> 3'AcTIR}..GUS->

25 independent barley lines (cv. Golden Promise) in which the GUS reporter gene is constitutively expressed have been recovered. These T0 transgenic plants have been crossed with barley lines expressing maize Ac transposase. Seed from these hybrid plants will be screened for transposition and for resistance to Puccinia hordei , barley leaf rust.
Several (>10) presumptive maize lines (cv. A188) containing the same Ds construct have been recovered and will be crossed by an Ac containing line to generate analogous material for screening for rust resistance in maize.
These transgenic cereal lines can also be used to recover over-expression mutants for a variety of morphological, biochemical, physiological and developmental phenotypes.

Detection of Maize MuDR Transposon of the Mutator Family in the Races Bolita and Zapalote Chico from Oaxaca (Mexico) as a Tool for Gene Flow Monitoring

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Mexico is a primary center of diversity for maize (Zea mays L.). It has been proposed that transposable elements have played a role in generating this diversity.
The maize Mutator transposons belong to one of the most active transposable element family described in any organism. Active Mutator generate high frequency of mutation. MuDR is the regulator element of the Mutator and has been discovered in the lines of Robertson (1978). MuDR has also been found in accessions of Zapalote chico race in Mexico (Gutierrez et al. 1998).
The objective of this study was to detect the presence of MuDR in the races Bolita and Zapalote chico from Oaxaca, and use it as an indication of gene flow between these two races. In our survey of farmers in the Central Valleys of Oaxaca we had detected that farmers exchange seed frequently between the Zapalote chico area and the Bolita area.
Our results are based on Southern blot and PCR analyses and indicate the presente of an intact, non methylated MuDR in 18 (71%) samples from the race Bolita and in 39 (72%) samples of the race Zapalote chico. The size of these MuDR is distinct from the standard MuDR, and is in agreement with the size described for the MuDR-Zc.
The samples of race Bolita from farmers that had introduced some Zapalote chico in their fields were found positive for the presence of MuDR. However, MuDR elements were also detected in many more samples. Our conclusion is that MuDR is already well distributed in many samples of maize from the Central Valleys of Oaxaca. It is therefore difficult to use this element as a marker for gene flow. However, these results strongly suggest that the MuDR element is much more broadly distributed than first reported. A survey of samples from other areas of Mexico would be helpful in defining the current distribution of this element in maize landraces.

Transposases Controlling Mutator Activity

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Mutator activity in maize is controlled by the transposable element *MuDR*. This autonomous element is composed of two convergently transcribed genes, *mudrA* and *mudrB*, separated by intergenic region and flanked by long terminal inverted repeats (TIRs). The *mudrA* gene encodes several forms of MURA transposase by means of alternative splicing. We have earlier demonstrated that alternative splicing of the first *mudrA* exon differentiates whether translation starts with ATG at position +450 resulting in MURA823 (or MURA736 when the last *mudrA* intron is retained) trasposase or, alternatively, ATG at position +224 could specify production of the novel MURA854 protein that has an alternative N-terminus. Interestingly, *in vivo* production of MURA854 would require a translational frameshift at the novel 3' acceptor site of the first *mudrA* intron at position + 466. To determine the functional role of each MURA protein *in vivo* we expressed them from the native TIR promoter in transgenic maize. In addition, to determine if translational frameshifting occurs, two MURA854 transgenes were constructed, one expressing a native cDNA (frameshift required) and the other is a frameshift corrected form. Both MURA823 and MUR736 transposases program somatic excisions of non-autonomous *Mu* elements from anthocyanin reporter alleles. However, no *Mu* insertion events have been observed, even when these MURAs are crossed into lines that express MURB protein(s). In contrast, we found that both MURA854 transgenes are capable of mediating both *Mu* element excisions and insertions, albeit at lower frequency when compared to *Mutator* lines. Current progress in characterization of MURA transposases will be presented. We hypothesize that alternative splicing and translational frame shift could represent key steps in regulation of

Mutator activity and that distinct forms of MURA transposase that exist *in vivo* might be functionally complementary.

Post-Transcriptional and Transcriptional Gene Silencing of the Mutator Transposable Element Family by Mu Killer

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Mu killer (Muk) is a dominant locus that heritably silences the autonomous Mutator family transposon, MuDR. The Muk-induced silencing of MuDR initiates very early, with effects on Mutator element methylation observed 22 days after pollination. Early in the silencing process, a specific class of small RNA (a hallmark of post-transcriptional gene silencing) homologous to the 5' end of the MuDR encoded transposase, mudrA, is found only in plants that carry both Muk and MuDR. Interestingly, this class of small RNA is observed only transiently: later in development, this class of small RNA is no longer present and mudrA switches from a partially methylated / post-transcriptionally silenced state to a fully methylated / transcriptionally silenced state. The other MuDR encoded gene, mudrB, is still transcribed in the same tissue where mudrA has become transcriptionally silent, but these mudrB transcripts are not polyadenylated. Likewise, Muk can efficiently silence a CaMV 35S:mudrA cDNA construct, suggesting that only the coding region of mudrA is required for silencing by Muk. In subsequent generations, both mudrA and mudrB are fully methylated and transcriptionally silent following the segregational loss of Muk. Conversely, Muk does not have a lasting effect on non-autonomous Mutator elements. These results suggest that the initiation of Muk-mediated silencing involves a mudrA-targeted post-transcriptional mechanism, but maintenance of the silenced state is transcriptional. Since the methylation of MuDR is both initiated during post-transcriptional silencing and maintained after the trigger for silencing (Muk) is lost, we suggest that Muk may be involved in the initiation of RNA-directed DNA methylation.

Finally, we report that Muk is competent to silence even the most active of high-copy Mutator tagging lines with 100% penetrance, suggesting it will be useful for those seeking to inactivate Mutator lines in which a mutation has been successfully recovered. More information on using Muk to silence active Mutator tagging lines is available at http://plantbio.berkeley.edu/~mukiller.

Development of a Promoter Trapping System in Zea mays L. Using the Transposable Element Mutator and Regulators of the Anthocyanin Biosynthesis

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The identification of tissue specifically or developmentally regulated promoter sequences is still of growing interest for the development of more efficient approaches in transgenic plant research. Therefore we are developing a promoter trapping system that utilizes the maize specific transposable element Mutator (Mu) as a tool for transposition. Two regulatory genes of the anthocyanin biosynthesis pathway, R and C1, are used as reporter genes. After transformation and transposition of the cassette by the autonomous transposable element MuDR, a tagged promoter activity will induce the anthocyanin biosynthesis regulator gene(s) in the trapping construct. The specific promoter activity should be visible by color formation in the specific plant tissues that are responsible for promoter activity. The suitability of the chosen reporter genes, was successsfully demonstrated in stably transformed plants. The over-

expression of each of the genes led to visible anthocyanin coloration in several different plant tissues. Plants obtained from crosses between C1 and R over-expressing lines showed strong anthocyanin coloration in most tissues. These experiments demonstrate that nearly all tissues of the maize plant are capable of color development after expression of both of the regulatory genes C1 and R. Therefore C1 and R fulfill the prerequisites to be suitable reporter genes in the system.
The cloning of a functional trapping construct will only be possible if the reporter gene can be expressed (although it is flanked by the two TIR of the transposable element) and if the reporter gene is not induced by a putative TIR specific promoter activity. To prove these conditions extensive transient and stable expression experiments with various TIR reporter gene constructs were carried out. It could be shown that the cloned TIR sequences possess no specific promoter activity. However, a reporter gene activity was reduced if a TIR sequence has been cloned between the promoter and the reporter gene. To optimize the read through capability, a point mutation was introduced into the TIR. The mutation enhanced the reporter gene activity more than threefold. Trapping constructs containing one or both of the reporter genes were cloned, tested in transient experiments and introduced into suitable maize lines by biolistic transformation. The obtained plants were crossed with a line carrying the autonomous Mutator element MuDR. PCR-analysis of the progenies proved transposase mediated insertion events of the trapping construct in these plants. The current results suggest a high transposition frequency of the trapping construct and the convenient use of the constructs in the achieved promoter trapping system.

The mop1 (Mediator of Paramutation1) Mutation Exhibits Maternally-Dependent Reactivation of Silenced Mutator Transposons in Maize

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The Mutator system in maize is a highly mutagenic class of transposons, consisting of the autonomous element MuDR and several nonautonomous elements which share ~220bp terminal inverted repeats (TIRs). MuDR encodes two genes: mudrA (the putative transposase) and mudrB (a helper gene). Mu killer, a single dominant locus in maize, is able to epigenetically and heritably silence MuDR. Aspects of MuDR silencing by Mu killer include the loss of transposition, the suppression of transposon excision (as exhibited by the loss of spotted kernels in the aleurone) and a simultaneous methylation of TIRs. The loss of activity is also associated with features of both transcriptional and posttranscriptional silencing of mudrA and mudrB. The mop1 mutation, which causes reversal of paramutation in the paramutagenic alleles of color genes such as pl1, b1, and r1, can also partially reactivate silenced Mutator elements: TIRs become hypomethylated, somatic excision is observed, and mudrA expression returns. However, mudrB expression does not reappear, and Mutator transposition is not restored. Given that mudrB is required for transposition but not excision, these data suggest that the mop1 mutation relieves silencing of only one of the two genes encoded by MuDR, a conclusion that is supported by the observation that the promoters of the two genes are differentially hypomethylated in mop1 mutants. Unlike silencing by Mu killer, Mutator reactivation by mop1 is not heritable. Furthermore, a high frequency of somatic excisions requires that the maternal genotype be mop1 mutant. In contrast, passage through the male gametophyte results in a heritable reduction is the level of somatic activity. Interestingly, half of the spotted kernels from a segregating family where the maternal genotype is mop1 mutant are genotypically mop1 heterozygous; conversely, spotted kernels from the exact reciprocal cross where the paternal

genotype is mop1 are always mop1 homozygous. These findings suggest that there are clear parent-of-origin effects with respect to the initiation and maintenance of reactivation in a mop1 mutant background.

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